FORMULATION DEVELOPMENT OF STABLE AMBROXOL HYDROCHLORIDE SYRUP AND COMPARATIVE EVALUATION WITH MARKETED SAMPLES

A dissertation submitted to

THE TAMIL NADU Dr. M.G.R MEDICAL UNIVERSITY CHENNAI,

In partial fulfilment of the requirements

For the award of the degree of

MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted by

BALAJI. P

Reg.No.26114502

Under the guidance of

Mrs.Rajrajeswari Hariharan,M.Pharm
Assistant professor
Department of Pharmaceutics
K K College of Pharmacy
Chennai-600122

Apr-2013

DEPARTMENT OF PHARMACEUTIC
K K COLLEGE OF PHARMACY
GERUGAMBAKKAM

CHENNAI-600122

CERTIFICATE

Prof. A.MEENA, M.Pharm, (Ph.D),

PRINCIPAL,

K.K. COLLEGE OF PHARMACY,

CHENNAI – 600 122.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF STABLE AMBROXOL HYDROCHLORIDE SYRUP AND COMPARITIVE EVALUATION WITH MARKETED SAMPLES"** is a bonafide and genuine research work carried out by **Mr. BALAJI. P ,** during the academic year 2012-2013 under the supervision of **Mrs. RAJRAJESHWARI HARIHARAN, M.Pharm.,** Professor, K.K. College of Pharmacy, Chennai - 600122. This dissertation submitted in partial fulfilment for the award of degree of **Master of Pharmacy (Pharmaceutics)** by The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

Signature of the Principal

Prof. Dr. V. VAIDHYALINGAM.,M.Pharm., Ph.D.

DIRECTOR

K.K. COLLEGE OF PHARMACY

CHENNAI – 600 122.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF STABLE AMBROXOL HYDROCHLORIDE SYRUP AND COMPARITIVE EVALUATION WITH MARKETED SAMPLES"** is a bonafide and genuine research work carried out by **Mr. BALAJI. P,** during the academic year 2012-2013 under the supervision of **Mrs.RAJRAJESHWARI HARIHARAN, M.Pharm.,** Professor, K.K. College of Pharmacy, Chennai - 600122. This dissertation submitted in partial fulfilment for the award of degree of **Master of Pharmacy (Pharmaceutics)** by The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

Signature of the Director

Prof. Dr. K. SENTHIL KUMARAN, M.Pharm., Ph.D.

HEAD OF DEPARTMENT

DEPARTMENT OF PHARMACEUTICS

K.K. COLLEGE OF PHARMACY

CHENNAI – 600 122.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF STABLE AMBROXOL HYDROCHLORIDE SYRUP AND COMPARITIVE EVALUATION WITH MARKETED SAMPLES"** is a bonafide and genuine research work carried out by **Mr. BALAJI. P ,** during the academic year 2012-2013 under the supervision of **Mrs.RAJRAJESHWARI HARIHARAN, M.Pharm.,** Professor, K.K. College of Pharmacy, Chennai - 600122. This dissertation submitted in partial fulfilment for the award of degree of **Master of Pharmacy (Pharmaceutics)** by The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

Signature of the Head of Department

Mrs.RAJRAJESHWARI HARIHARAN, M.Pharm,.

PROFESSOR,

INSTITUTIONAL GUIDE

DEPARTMENT OF PHARMACEUTICS

K.K. COLLEGE OF PHARMACY

CHENNAI – 600 122.

CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION DEVELOPMENT OF STABLE AMBROXOL HYDROCHLORIDE SYRUP AND COMPARITIVE EVALUATION WITH MARKETED SAMPLES"** is a bonafide and genuine research work carried out by **Mr. BALAJI. P,** during the academic year 2012-2013 under my supervision. This dissertation submitted in partial fulfilment for the award of degree of **Master of Pharmacy (Pharmaceutics)** by The Tamil Nadu Dr. M.G.R Medical University, Chennai – 600032.

Signature of the Guide

ACKNOWLEDGMENT

ACKNOWLEDGMENT

The work of some unknown person makes our lives easier every day. I believe it is appropriate to acknowledge all of those unknown persons, but it is also necessary to acknowledge those people who have directly shaped my lives and work.

Let me first thank almighty for giving me life and my parents for educating me and keeping my requirements in priority at all situations. Without their unconditional support and encouragement it was impossible to pursue my interest.

I am deeply indebted to ***Prof. K.R.ARUMUGAM, M.Pharm, CHAIRMAN, K.K.COLLEGE OF PHARMACY, CHENNAI*** *for his help and support.*

I wish to express my deep sense of gratitude and sincere thanks to ***Mrs.A.MEENA, M.Pharm, PRINCIPAL, K.K.COLLEGE OF PHARMACY, CHENNAI*** *for her help and support throughout my work.*

Now I take this opportunity to express my deep gratitude to ***Prof. Dr. V.VAIDHYALINGAM, M.Pharm., Ph.D., DIRECTOR K.K.COLLEGE OF PHARMACY, CHENNAI*** *for his help and support throughout these two years of my post graduation course. I would like to thank him for his hearty cooperation and valuable guidance throughout my M.Pharm course.*

I owe a debt of gratitude to ***Prof. Dr. K.SENTHILKUMARAN, M.Pharm., Ph.D., HEAD, DEPARTMENT OF PHARMACEUTICS*** *for his valuable guidance and providing facilities during the course of my work.*

I Profusely express my undying gratitude towards my guide ***Mrs.RAJARAJESWARI HARIHARAN, M.Pharm., Assistant Professor, Department of Pharmaceutics, K.K.COLLEGE OF PHARMACY*** *who stood by me throughout the project work and for spending her valuable time for giving me knowledge, encouragement and helping me for successful completion of my research work.*

I am deeply indebted to the teaching staffs of the department who were always a source of knowledge and inspiration to me. I would like to thank ***Miss.Kavitha, M.pharm., Mrs.PREETHA, M.Pharm.,*** *for their guidance and prompt assistance and cooperation.*

I express my sincere thanks to ***Mr.Saravanan, R&D Department, MMC Health care Limited, Thirumazhisai, Chennai-24.*** *for his valuable guidance, dynamic approach, innovative advices, technical and moral support given to me throughout the course of this dissertation work.*

I would like to thank ***Mr. Sathyamoorthy, Manager, QA department, and Mr. Bakyaraj, Manager, QC department, MMC Health care Limited, Thirumazhisai, Chennai-24.*** *for giving me the opportunity to work in this esteemed organization.*

I wish to express a special thanks to ***my beloved wife Mrs.B.MANJU and my family members.***

I express my special thanks to the Librarian and other non teaching staffs especially ***Mrs. Jamuna,*** *for their constant help and support through these two years of M.Pharm course.*

I take this opportunity to thank my friends ***K.SASIDHARAN, R.SUGI PAPPA*** *for their support and encouragement.*

I would to thank my batch mates ***H.ARUN A.SENTHIL KUMAR, A.AMUL RAJ,*** *for their support and encouragement throughout my project work.*

I would like to thank all my classmates, friends and to all those helped me in making this work.

THANKS TO ALL.....

BALAJI.P

ABBREVATIONS

SI.NO	SYMBOLS	ABBREVATIONS
1	ºC	Degree Celsius
2	%	Percentage
3	API	Active pharmaceutical ingredient
4	BP	British Pharmacopoeia
5	CFU	Colony forming unit
6	FT-IR	Fourier transform Infra-red spectroscopy
7	g	Gram
8	g/cm	Gram/centimetre
9	HCl	Hydrochloride
10	HPLC	High performance liquid chromatography
11	HPMC	Hydroxyl propyl methyl cellulose
12	ICH	International conference on harmonization
13	JP	Japanese Pharmacopoeia
14	KBr	Potassium bromide
15	µ	Micron
16	M	Molar
17	µg/ml	Microgram/millilitre
18	mg/ml	Milligram/ millilitre
19	ml	Millilitre
20	mPas	MilliPascal
21	MMC	Madras Medical College Pharma
22	MSA	Mannitol salt agar media
23	NCC	No characteristic change
24	nm	Nanometre
25	Ph Eur	European Pharmacopoeia
26	PVP	Polyvinyl Pyrrolidine
27	RH	Relative humidity
28	SCA	Sabouraud chloramphenicol agar medium
29	SCDA	Soyabean casein digest agar medium

30	TLC	Thin-layer chromatography
31	UV	Ultra violet
32	USP	United States Pharmacopeia
33	w/w	Weight/weight
34	w/ml	Weight/millilitre

LIST OF TABLE

LIST OF FIGURE

CONTENTS

1.INTRODUCTION

DOSAGE FORMS

THE NEED FOR DOSAGE FORMS [1]

The potent nature and low dosage of most of the drugs in use today precludes any expectation that the general public could safely obtain the appropriate does of drug from the bulk material. Most drug substances are administered in milligram quantities, much too small to be weighed on anything but a sensitive prescription or electronic analytical balance.

When the dose of the drug is minute, solid dosage forms such as tablets and capsules must be prepared with fillers or diluents so that the dosage unit is large enough to pick up with the fingertips.

Besides providing the mechanism for the safe and convenient delivery of accurate dosage, dosage forms are needed for additional reasons.

- To protect the drug substance from the destructive influences of atmospheric oxygen or humidity (coated tablets, sealed ampoules)
- To protect the drug substance from the destructive influence of gastric acid after oral administration (enteric-coated tablets).
- To conceal the bitter, salty, or offensive taste or odor of a drug substance(capsules, coated tablets ,flavored syrups)
- To provide liquid preparations of substances that are either insoluble or unstable in the desired vehicle (suspensions)
- To provide clear liquid dosage forms of substances (syrups, solutions)
- To provide rate-controlled drug action (various controlled-release tablets, capsules and suspensions)
- To provide optimal drug action from topical administration sites (ointments, creams, transdermal patches and ophthalmic, ear and nasal preparations)
- To provide for insertion of a drug into one of the body's orifices(rectal or vaginal suppositories)

- To provide for placement of placement of drugs directly in the bloodstream or body tissues (injections)
- To provide for optimal drug action through inhalation therapy (inhalants and inhalation aerosols)

GENERAL CONSIDERATIONS IN DOSAGE FROM DESIGN

Before formulating a drug substance into a dosage from, the desired product type must be determined as far as possible to establish the framework for product development. The formulation that best meets the goals for the product is selected to be its master formula. Each batch of product subsequently prepared must meet the specifications established in the master formula.

These liquids, which are flavored aqueous solutions, syrups or suspensions are usually administered directly into the infant's or child's mouth by drop, spoon or oral dispenser or incorporated into the child's food. A single liquid pediatric preparation may be used for infants and children of all ages, with the dose of the drug varied by the volume administered. Infant-size rectal suppositories may also be employed, although drug absorption from the rectum is often erratic.

Some medications are formulated as chewable tablets. Newly available tablets dissolve in the mouth in about 10 to 15 seconds. This allows the patient to take a tablet but actually swallow a liquid.

Medications intended for the elderly are commonly formulated into oral liquid or may be extemporaneously prepared into an oral liquid by the pharmacist.

Types of dosage forms: [2]

There are different forms into which a drug may be placed (regulated/given or taken) for convenient and effective treatment of disease. Drugs can be prepared for administration by very conceivable route and the suitable preparation is formulated to insure maximum therapeutic response. These may be tablets, capsules, solutions, syrups, elixirs, suspensions, gels, powders, troches or lozenge , ointments, creams, pastes, aerosol, lotions, sprays, inhalants, emulsions and suppositories .The preferred dosage forms in different routes of administration is listed.

Table No.1 Types of dosage form

S.No	TERM	SITE
1	Oral	Tablets, Capsules, Solutions Syrups, Elixirs, Suspensions Gels, Powders.
2	Sublingual	Tablets Troches or Lozenge
3	Parenteral	Solutions Suspensions
4	Epicutaneous	Solutions, Suspensions Gels, Powders Ointments, Sprays.
5	Conjunctival	Ointments and Solutions
6	Intraocular	Solutions and Suspensions
7	Intranasal	Solutions, Ointments Aersol, Sprays Inhalants
8	Intrarespiratory	Aerosol
9.	Rectal	Solutions, Ointments Suppositories
10	Vaginal	Tablets, Solutions, Ointments, Emulsions and Suppositories
11	Urethral	Solutions and Suppositories

Advantages and Disadvantages of oral route of administration: Drugs are usually taken by oral route of administration. The advantages and disadvantage of oral route of administration are discussed below:

Advantages of oral route of administration: [3]

1. It is most easy route for administration of drug for patients.
2. It is the safest route of administration.
3. It is most convenient for patients.
4. This route can be used for large variety of dosage forms.
5. Nursing for administration is not required.
6. An allergic reaction of drug (toxicity) is delayed and hence safe.
7. It is economical to the patients.

Disadvantages of oral route of administration:

1. The onset of action of drug is late and hence recovery is not fast. Therefore oral route of administration is not preferred in emergency.

2. As it is absorbed form gastrointestinal tract the dose of drug required is more.

3. It is difficult route of administration of drug for non-cooperative patients like babies and children.

4. It is difficult route of administration of drug for unconscious patients.

5. The absorption of drug from gastrointestinal tract is not assured by patients suffering from gastrointestinal disorder.

6. Oral route of administration may cause gastrointestinal disorders like acidity, loss of appetite, etc.

7. The uncertainty of maintenance of the dose of drug is possible in oral route of administration.

8. Drug may be destroyed or inactivated by the enzyme in gastrointestinal tract.

LIQUID DOSAGE FORMS[4]

INTRODUCTION:

Solution is a homogenous one-phase system consisting of two or more components. The solvent, or mixture of solvents, is the phase in which the dispersion occurs, and the component which is dispersed as molecules or ions in the solvent.

For most pharmaceutical solutions, the solvent system is likely to be liquid, and the solute will be either a liquid or a solid. Solid dispersions, in which both solute and solvent are solids, are used for the improvement of the bioavailability of poorly soluble drugs. The solute is present as a molecular dispersion and will therefore exhibit a fast rate of dissolution, owing to its very high specific surface area. In addition, the particles are already in a disaggregated and wetted state, and so there is little air adsorbed on the particle surface to inhibit dissolution. There may even be slight increases in actual solubility.

Solutions are used in many ways; they are-

a) Taken orally—as mixtures, elixirs, linctuses, draughts, syrups and paediatric drops.

b) Used in the mouth and throat- as mouthwashes, gargles, throat paints and throat sprays.

c) Instilled into body cavities- as douches, enemas, ear drops, nasal drops nasal sprays.

d) Applied to body surfaces- as collodions, liniments, lotions and paints.

Solutions may be prepared from any combination of solid, liquid and gas, the three states of matter. Solutions are liquid preparations that contain one or more chemical substances dissolved in a suitable solvent or mixture of mutually miscible solvents.

Oral solutions, syrups, elixirs, spirits and tinctures are prepared and used for the specific effects of the medicinal agents they carry. In these preparations, the medicinal agents are intended to provide systemic effects. The fact that they are administered in solution form usually means that they are soluble in aqueous systems and that their absorption from the gastrointestinal tract into the systemic circulation may be expected to occur more rapidly than from suspension or solid dosage forms of the same medicinal agent.

Solutes other than the medicinal agents are usually present in orally administered solutions. These additional agents usually are included to provide color, flavor, sweetness, or stability.

Advantage of Liquid dosage forms:

- Effective more quickly than a solid dosage because drug is already dissolved in a liquid.
- Easier to swallow than solid dosage form for many patients.
- Drugs may be available only in liquid form owing to convenience of administration.
- Uniformity and flexibility of dosage form.
- Certain medications may cause gastrointestinal distress if administered in a solid dosage form.

Disadvantages of Liquid dosage forms:

- Deterioration and loss of potency occur more quickly than solid dosage form.
- Incompatibilities of dissolved substances.
- May require preservatives to prevent bacteria or mold from developing.
- Inaccurate measuring of a dose for patient may occur.
- Interactions may develop from changes in solubility.
- Bulkier and inconvenient to carry than solid dosage forms.

SYRUPS[5]

Syrups are concentrated, aqueous preparations of a sugar or sugar-substitute with or without added flavoring agents and medicinal substances. Syrups containing flavoring agents but not medicinal substances are called flavored vehicles (syrups).e.g. Cherry Syrup, Cocoa Syrup, Orange syrup, Raspberry Syrup. Syrups containing medicinal agents are called medicated syrups. e.g. Chlorpheniramine maleate syrup, Ipecac syrup, Chloral hydrate syrup etc.

Components of syrups:

Most syrups contain the following components in addition to the purified water and any medicinal agents present:

1. The sugar, usually sucrose, or sugar substitutes used to provide sweetness and viscosity,
2. Antimicrobial preservatives, Flavorants, and Colorants.

Sucrose and non-sucrose based syrup:

Sucrose is most frequently employed in syrups. In special circumstances it may be replaced by sugars, such as, dextrose, or non-sugars as sorbitol, glycerin and propylene glycol.

Methyl cellulose or hydroxyethyl cellulose -these two materials are not hydrolyzed and absorbed into the blood stream and their use results in an excellent syrup-like vehicle.

Taste masking by syrup:

The syrup imparts a characteristics "body" (viscosity) and together with the sweetness and the flavorants results in a type of pharmaceutical preparation that is quite effective in masking the taste of added medicinal agents. When the syrup is swallowed, only a portion of dissolved drug actually makes contact with the taste buds, the remainder of the drug being carried past them and down the throat in the containment of the viscous syrup. In the case of antitussive syrups (e.g. linctus) the thick sweet syrup has a soothing effect on the irritated tissues of the throat as it passes over them.

Preparation of Syrups:

Syrups are frequently prepared by one of four general methods. depending upon the physical and chemical characteristics of the ingredients.

1. Solution of the ingredients with the aid of heat

2. Solution of the ingredients by agitation without the use of heat

3. Addition of sucrose to a prepared medicated liquid or to a flavored liquid and

4. By percolation of either the source of the medicating substance or of the sucrose.

Solution with the aid of heat:

The sugar is generally added to the purified water, and heat is applied until solution is effected. Then other required heat-stable components are added to the hot syrup, the mixture is allowed to cool, and its volume is adjusted to the proper level by the addition of purified water. The use of heat facilitates the rapid solution of the sugar as well as certain other components of syrups. If excessive heating occurs then sucrose may be hydrolyzed into dextrose (D-glucose), and fructose (levulose). This hydrolytic reaction is referred to as inversion, and the combination of the two monosaccharides is invert sugar.

When heat is applied in the preparation of sucrose syrup, some inversion of the sucrose is almost certain. The speed of inversion is greatly increased by the presence of acids, the hydrogen ion acting as a catalyst to reaction. Invert sugar is sweeter than sucrose, and normally colorless. Syrup darkens due to the effect of heat on the fructose. When the syrup is

greatly overheated, it becomes amber colored due to the caramelization of the sucrose. Syrups so decomposed are more susceptible to fermentation and microbial growth. Because of the prospect of decomposition by heat, syrups cannot be sterilized by autoclaving.

Solution by agitation without heat:

Sucrose and other formulation agents may be dissolved in purified water by placing the ingredients in a vessel of greater capacity than the volume of syrup to be prepared, thus permitting the thorough agitation of the mixture.

Advantage of syrups:

- The active agent is homogeneously dispersed through the product.
- The active agent is in solution and does not need to undergo dissolution ; therefore , the therapeutic response is generally faster than if a tablet or capsule dosage from is used for treatement.
- The dose of the active agent is easily and conveniently adjusted by measuring a different volume.
- Syrups may be swallowed by patients who have difficulty taking tablets or capsules, as might be thease with pediatric or geriatric patients.
- Drugs such as potassiumchloride that may casue ulceration to the mucosa in a tablet formulation avoid this side effect when present in solution.

Disadvantage of syrups:

- The active ingredients, when present in solution, are usually more susceptible to chemical degradation, particualry hydrolysis, than when they are in solid dosage form.
- As a consequence of this, the soultion product has a shorter shelf life than the soild formulation.
- Some pharmacologic agents taste or smell bad enough in solution that the patient has difficulty taking the medication.
- Liquid dosage forms are heavier and take up more shelf space than corresponding soild dosage sorms. If the container breaks, the porduct is irretrievably lost.

- Liquid dosage forms may require special storage facilities in very cold or very hot conditions. One example of this involves taking, medicine , such as an antibiotic suspension , on a lengthy trip. In one case the drug might need to be kept refrigerated, and in another case the patient may need to protect the drug from freezing.
- The delivery of the dose depends upon the patient , or care-giver , measuring the proper volume.This can be a significant issue for vision-impaired patients , patients with arthritis, or patients unable to read the numbers on an oral dosing syringe or medicine cup.
- Liquid preparation are often susceptiable to microorganisms, and therefore preservatives are frequently incorporated into the formulation .Some patients may be allergic to certain preservatives.

TASTE MASKING

Swallowing tablets is a problem for many patients particularly for children and geriatric patients especially when the tablets are large. Certain medicaments have an unpleasant taste in the throat when they are orally administrated. So some agents are added disclosed which when incorporated in the composition mask these bitter taste .The conventional oral dosage forms possess sustained release anti-tussive characteristics.

Microcapsules are formulated into chewable taste masked oral tablets or capsules that provide for immediate rapid release in the stomach. The meth acrylic acid copolymer can be a copolymer of polymethacrylic acid and acrylic acid esters. These polymers coating should be used for immediate release characteristics in microcapsule techniques.

Many drugs containing amine or amide groups or salts there of often have a strong bitter taste. Taste masking techniques using various sweeteners, amino acids, flavors & adsorbents have been unsuccessful in masking the taste. Most coating techniques don't have an acceptable in-vivo drug releasing mechanism cation exchange resins have been used to adsorb amine drugs for sustained release action & taste masking. The widely used cat ion-exchange resins are poly sulfonic acid & poly carboxylic acid polymers.

A variety of delivery systems are being developed for different routes of administrations like oral, parenteral, nasal & transdermal, The oral route remains attractive

for drug delivery because this mode of administration is an easy, convenient, noninvasive & familiar method of drug delivery .

The common oral dosage forms include liquid mixtures, tablets, capsules & liquid filled capsules. The solid dosage forms are futher modified depending on the therapeutic action desired like controlled, extended, or delayed release.

Patients at the extremes of age, such as children and the elderly often experienced difficulty in swallowing solid dosage forms. Liquid dosage forms such as solution, emulsions & suspensions. Usually lead to perceptible exposure of the active drug ingredient to the taste buds, which is a very serious problem when the drug has an extremely unpleasant taste.

Conventional taste masking techniques such as sweeteners, amino acids, flavoring agent are often unsuccessful in masking the taste of the highly bitter drugs like quinine, ofloxacin & clarithromycin etc.

Taste masking is a major problem when the drugs are extremely unpleasant. This problem is not restricted to the liquid oral composition like solution, dry syrup & suspensions but may also be encountered during the formulation of chewable tablets where in these dosage forms usually lead to perceptible exposure of active ingredient to taste buds. Depending on the type of dosage form various methods have been employed to overcome the unpleasant taste and bitterness of the drug.

Various method for taste masking have been tried earlier which include use of ion exchange resins, complexation of bitter drugs with pharmaceutically acceptable recipients and coating of drugs by lipids using a various polymeric materials. Coating of the active ingredient can be done by any of the techniques known in the art like microencapsulation.

The use of ion exchange resins to adsorb drugs containing amino groups for taste masking has found limited applicability in masking the taste of highly bitter drugs, and also where the drugs is to be dispersed in a liquid oral composition for long duration of time.

Another technique is mouth disintegrating tablet. In this method, The tablet disintegrate in oral cavity with saliva in 15 sec to 60 sec, without need of water and should have pleasant mouth feel.

These task masking formulations frequently are flavored with fruit or mint flavors, usually for purposes of masking an unpleasant taste caused by the presence of a dissolved or suspended pharmacologically active substance. A pleasant taste is particularly important when the formulation is intended for ingestion by children. The typical flavors which are commonly used in formulations. They are grape, cirus, peach, strawberry, peppermint and many other flavors.

TECHNIQUES EMPLOYED FOR TASTE MASKING

The methods commonly employed for achieving effective taste masking include various physical and chemical methods that prevent the drug substance from interaction with the taste buds.

A) Use of Flavor Enhancers

The materials for taste masking purpose have often been classified depending upon the basic taste that is masked. Flavoring and perfuming agents can be obtained from either natural or synthetic sources. Natural products include fruit juices, synthetic products like aromatic oils such as peppermint oil and lemon oil, herbs, spices and distilled fractions. They are available as concentrated extracts, alcoholic or aqueous solutions, syrups or spirit. Apart from these conventional materials many compositions have been found to show effective taste masking abilities with improved flavor such as alkaline earth oxide, alkaline earth hydroxide or an alkaline hydroxide. Another composition includes phosphorylated amino acid such as phosphotyrosine, phosphoserine, and phosphothreonine and mixtures thereof. Anethole effectively masked bitter taste as well as the after taste of zinc, which is used in treating the common cold. Clove oil and calcium carbonate which has been found to be particularly useful to mask the unpalatable taste in formulation which are intended to be chewed or dissolved in mouth prior to ingestion.

B) Applying Polymer Coatings

Coating of drugs using a suitable polymer offers an excellent method of concealing the drug from the taste buds. The coated composition may be incorporated into number of pharmaceutical formulations including chewable tablet, effervescent tablets powder and liquid dispersions. Multiple encapsulated flavor delivery systems has been developed which is useful in chewing gum, pharmaceutical preparations as well as other food products.

C) Complexation and Adsorption Approaches

Complexation with ion exchange Resins and Polymers

Cat ion-exchange resin CRP 244 and anion exchange ion were used to adsorb ester drugs for both masking of bitter taste and achieving sustained release action. The types of ion exchange resins that have been successfully used to mask the taste of bitter drugs include amberlite IRP 88 (an acrylic potassium resin). amberlite IRP 64 (a polystyrene sulphonate) and amberlitr IRP 64 (a carboxylate form of the methapyrilene, dextromethrophan, ephedrine and pseudeuphedrine) were masked by first forming adsorbates with polymethacrylic acid ion exchange resin followed by coating of resin complex with 4:1 mixture of ethyl cellulose and hydroxyl propyl methyl cellulose (HPMC) polymers.

Taste evaluation of the adsorbents showed a significant reduction in the bitterness of the drugs. Coating adsorbent particles with 3:1 ethyl cellulose – HPMC mixture reduced the bitterness further. Taste coverage was maintained after incorporation of the coated adsorbent in to chewable tablets. Strong acid cat ion resins (sulfonated styrene divinyl benzene copolymer product) can be used for masking the taste of basic drugs.[23] Polystyrene matrix cat ion exchange resins have been used to mask the bitter taste of chlorpheniramine malate, ephedrine hydrochloride and diphenhydramine hydrochloride.[24] Extreme bitterness of quinolones has been achived by ion exchange resin such as methacrylic acid polymer cross linked with divinylbenzene.

FORMULATION OF SYRUPS

The major components of syrups are as follows:

- Purified water
- Sugar (sucrose) or sugar substitutes (artificial sweeteners).

Traditionally syrups are composed of sucrose (usually between 60 and 80%) and purified water. Due to the inherent sweetness and moderately high viscosity of these systems, the addition of other Sweetening agents and viscosity-modifying agents is not required. In addition, the high concentration of sucrose and associated unavailability of water (termed low water activity) ensures that the addition of preservatives is not required. As the

concentration of sucrose is reduced from the upper limit (e.g. through dilution), the addition of preservatives may be required.

In some formulations, other non-sucrose bases may replace traditional syrup. One of the most popular is Sorbitol, which contains 64% w/w sorbitol , although other alternatives are available that are based on mixtures of sorbitol and glycerin. These non-sucrose bases may be mixed with traditional syrups, if required, in the formulation of oral syrups that possess a low concentration of sucrose in comparison to traditional syrups.

More recently, many products have been formulated as medicated sugar-free syrups due to the glycogenetic and cariogenic properties of sucrose. For the afore-mentioned reasons, all medicinal products designed for administration to children and to diabetic patients must be sugar-free.

Sugar free substitutes must therefore provide an equivalent sweetness, viscosity and preservation to the original syrups. To achieve these properties artificial sweeteners (typically saccharin sodium, aspartame), non-glycogenetic viscosity modifiers (e.g. methylcellulose, hydroxyethylcellulose) and preservatives (e.g. sodium benzoate, benzoic acid and parahydroxybenzoate esters) are included.

■ **Preservatives:** Preservatives are not required in traditional syrups containing high concentrations of sucrose. Conversely, in sugar-free syrups, syrups in which sucrose has been substituted at least in part by polyhydric alcohol and in traditional syrups that contain lower concentrations of sucrose, the addition of preservatives is required.

■ **Flavours:** These are employed whenever the unpalatable taste of a therapeutic agent is apparent, even in the presence of the sweetening agents. The flavours may be of natural origin (e.g. peppermint, lemon, herbs and spices) and are available as oils, extracts, spirits or aqueous solutions. Alternatively, a wide range of synthetic flavours are available that offer advantages over their natural counterparts in terms of purity, availability, stability and solubility. Certain flavours are also associated with a (mild) therapeutic activity. For example, many antacids contain mint due to the carminative properties of this ingredient. Alternatively other flavours offer a taste-masking effect by eliciting a mild local anaesthetic effect on the taste receptors. Examples of flavours in this category include peppermint oil, chloroform and menthol. The concentration of flavour in oral syrups is that which is required to provide the required degree of taste-masking effectively.

■ **Colours:** These are generally natural or synthetic watersoluble, photo-stable ingredients that are selected according to the flavour of the preparation. For example, mint-flavoured formulations are commonly green color, whereas in banana-flavored solutions a yellow colour is commonly employed. Such ingredients must not chemically or physically interact with the other components of the formulation.

2. AIM AND OBJECTIVE

Aim of study:

- To formulate Ambroxol hydrochloride syrup by using different sweetening agents like sucrose , sucralose and sodium saccharin
- To evaluate the various formulations and to select the ideal formulation..
- To perform accelerated stability studies for all the formulations.
- To compare the best formulation with marketed tablet and syrup.

3. PLAN OF WORK

The study was planned as follows

- Literature survey
- Preformulation study of drug
- Preparation of different formulations.
- Evaluation of various parameters such as
    1. pH
    2. Viscosity
    3. Specific gravity
    4. Assay
    5. Dissolution study
    6. Microbial testing
    7. Related substances
- Stability testing of different formulations

4. LITERATURE REVIEW

Maheshwari R K et al., [6] formulated the syrup of poorly water-soluble drug Tinidazole. For the experiment, the blends containing solubilizers from the category of hydrotropes, co solvents and water soluble solids were employed. The blends of randomly selected solubilizers were used for solubility studies. Based on the solubility studies, few blends showing largest solubilites were employed to make the syrup. This may reduce the individual concentration of solubilizers and so reduce their potential of toxicities. The formulated syrups were subjected to accelerated stability studies and they were found to be quite stable.

Rajagopalan R et al.,[7] formulated the syrup of poorly water-soluble drug Paracetamol. For the experiment, the blends containing solubilizers from the category of hydrotropes, co solvents and water soluble solids were employed. The blends of randomly selected solubilizers were used for solubility studies. Based on the solubility studies, few blends showing largest solubilities were employed to make the syrup. This may reduce the individual concentration of solubilizers and so reduce their potential of toxicities. The formulated syrups were subjected to accelerated stability studies and they were found quite stable.

Ferreira D C et al.,[8] compared the stability of Ambroxol HCl syrup without sugar prepared in their pharmacy with two commercial preparations containing sugar. The bottles were filled with 50ml of syrup containing 6mg/ml Ambroxol HCl. The bottles were stored at 40ºC, 50ºC and 60ºC. Before filling and immediately after filling and at intervals during 75 days of storage, samples were taken and analysed by HPLC for Ambroxol HCl. The prediction of their syrup formulation shelf life was carried out by the accelerated stability programme.

Abdelkawy M et al.,[9] developed three simple, reliable methods for the simultaneous determination of Ambroxol HCl and Guaifenesin in the presence of oxidative degradants. The three methods used were isocratic HPLC method, TLC-spectrodensitometric method and multivariate spectrophotometric method. The methods were applied for pharmaceutical dosage forms containing either ambroxol alone (drops, capsules)and ambroxol together with guaifenesin syrup They have concluded that Multivariate calibration method was better method when compared to others for syrup dosage form due to interfering additives.

Stephen childs[10] presented a review on maple syrup. In the review he has given about crystallization, how to control crystallization and crystallization inhibitors, invert sugar, advantages, Maillard reaction etc.,

Patel Divyakant A et al.,[11] formulated and evaluated herbal syrup from the leaves of *Neolamarckia cadamba* (Roxb.) Bosser and have concluded that the syrup they have developed has unique position in development of new formulations.

Khan hajera et al.,[12] Developed and validated a dissolution test for Gemifloxacin mesylate and Ambroxol hydrochloride tablets using spectrophotometric method. The established dissolution conditions were: 900ml of 0.01M Hcl (pH2.0) as dissolution medium, using paddle apparatus at a stirring rate of 50rpm. The drug release was evaluated by UV spectrophotometric method at 271nm for Gemifloxacin mesylate and 243.5nm for Ambroxol hydrochloride. The method was validated to meet requirements for a global regulatory filing which includes linearity, specificity, precision, accuracy, robustness and ruggedness.

Joseph H B et al.,[13]Prepared syrups from orange juice serum and were tested for stability against microbial and chemical degradation. Syrups containing 70% soluble solids did not support growth of osmophilic yeasts, while pure sugar syrups prepared at 70ºBrix maintained cultures of yeasts for 35 days at 30ºC. Therefore orange syrups appear to contain microbial inhibitors. Nonenzymic browning of orange syrups was measured at 30ºC. Browning rates were lower when syrups were measured at 30ºC. Browning rates were lower when syrups were prepared from serum treated with cation exchange resin to remove amino acids and lower pH. Browning rates were higher when syrups were prepared from this serum after the pH was adjusted to 4.5.

Abdul A S et al.,[14] developed Ambroxol pellets to present in the form of capsules (Modified release capsules). Ambroxol coated pellets were formulated by using commercially available pellets and the capsules were filled by capsule filling machine. The accelerated stability studies were done for one month. They have concluded that the developed formulation has minimum volume in size, greater surface area and more surface disintegration time for pellets in capsules. Small volumes of pellets enter into the systemic circulation very fast. Moreover no accumulation of drug in the body occurs

Mizanur R M et al.,[15] prepared Ambroxol HCl sustained release matrix tablets by response surface methodology. 2^3 factorial design was used for the preparation of tablets. They have concluded that optimization of Ambroxol HCl by Response Surface Methodology is quite efficient.

Brooks A A et al.,[16] Examined five different types of high sugar paediatric syrups for bacteriological status. A second line investigation was also conducted on samples which did not show any sign of bacterial growth during the first trial. All the products gave low counts of contaminating bacteria when they were diluted directly, plated out on conventional solid media and colonies counted. Higher counts were obtained when the cells were reactivated and plated on hypertonic mannitol salt agar. They have concluded that the presence of some bacteria is indicative of unwholesome products which may have serious health implications in neonates and children.

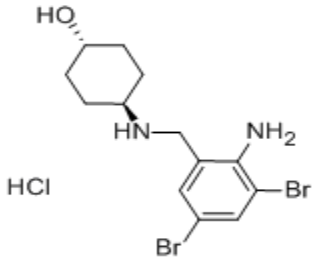
Priya c Jain et al.,[17] formulated taste masking Ambroxol Hydrochloride microspheres using spray drying technique using HPMC and PVP. The percentage yield was found to be 28.23% and the encapsulation efficiency was found to be 50.8%. the *in-vitro* drug release was found to be 70.39% in 0.1N HCl and pH 6.8 phosphate buffer solution.

Anbu Jeba Sunilson J et al.,[18] formulated and evaluated polyherbal cough syrup.The aqueous ethanol extracts of various traditional herbs like *Adhatoda vasica, Acorus calamus, Glyzyrrhiza glabra, Ocimum sanctum, Tylophora asthmatica, Piper longum* and *Solanum xanthocarpum* was evaluated for its antihistaminic activity by the inhibition of histamine induced contractions on the guinea pig ileum. The results showed that the formulated cough syrup inhibited histamine induced contractions of guinea pig ileum at 2.5 to 25 μg/ml concentrations in a dose dependent manner ($p < 0.01$, $p < 0.05$) and also significantly ($p < 0.05$) inhibited degranulation of mast cells. All the results were well comparable with the standard benadryl cough syrup (diphenhydramine).

5. DRUG PROFILE[19]

DRUG PROFILE

AMBROXOL HYDROCHLORIDE [18]



Molecular formula : $C_{13}H_{18}Br_2N_2O.HCL$

Chemical name : Trans4-2 anino-3, 5 dibromo benzyl amino Cyclohexanol hydrochloride

Appearance **:** A white or almost white crystalline powder odorless or almost odorless.

Solubility **:** soluble in methanol

N, N –dimethyl formanide

Slightly soluble in water and ethanol

Practically insoluble in chloroform and benzene

Storage **:** ambroxo hydrochloride should be protected from light.

Therapeutic category: Expectorant: enhanced mucolytic

Respiratory disorders: *Used in a variety of respiratory disorders including chronic bronchit

* Cystic fibrosis and infant respiratory distress syndrome .Also used in the treatment of cough.

Uricosuric action **:** It also shows uricosuric effect. The minimum effective does of lowering plasma uricacid concentrations was found to be between 250 mg and 500 mg daily given in 2 divided doses. Although these doeses are much higher than those used to treat broncho pulmonary disease, doses as high as 1 gram daily were well tolerated.

Daily dose **:** 30 to 120 mg has been given by mouth in 2 to 3 divided doses.

Adverse effect **:** hyper sensitivity a report of contact allergy to Ambroxol.

Mechanism of action **:** Ambroxol hydrochloride is a potent mucolytic and mucokinetic capable of inducing bronchial secretion. It is particularly useful in it mucus plugs are present.

Pharmacokinetics

Ambroxol hydrochloride is rapidly absorbed from the gastrointestinal tract and under goes extensive first pass metabolism in the liver .It is widely distributed to body tissues .About 85% of the drug is excreted as metabolites .It is highly bound to plasma proteins. It has a terminal half life of about 12 hours.

Ambroxol hydrochloride crosses the blood brain barrier and small amounts cross the placenta. Administration of Ambroxol hydrochloride by mouth to healthy subjects produce peak plasma concentration after about 1hour .Only small amounts were excreted unchanged in the urine with a half –life of about 6.5 hours.

Precautions[20]

It is advisable to avoid use during the first trimester of pregnancy.

Ambroxol Dosage

Adults : Daily dose of 30 mg (one Ambroxol tablet)to 120 mg (4 Ambroxol tablets) taken in 2 to 3 divided doses.

Children up to 2 years : Half teaspoonful Ambroxol syrup twice daily

Children 2 - 5 years : Half teaspoonful Ambroxol syrup 3 times daily

Children over 5 years : One teaspoonful Ambroxol syrup 2-3 times daily.

Storage : Store at a temperature not exceeding 30 degrees celsius. Keep all medicines out of reach of children.

Ambroxol Indications

All forms of tracheobronchitis, emphysema with bronchitis pneumoconiosis, chronic inflammatory pulmonary conditions, bronchiectasis, bronchitis with bronchospasm asthma. During acute exacerbations of bronchitis it should be given with the appropriate antibiotic.

Ambroxol Contraindications

There are no absolute contraindications but in patients with gastric

Brand Names: Mucosolvan and Mucoangin

Ambroxol is used for infections of the upper respiratory tract. It clears airways and eases cough. It enhances pulmonary surfactant production and stimulates ciliary activity. These actions result in improved mucus flow and transport (mucociliary clearance). Improvement of mucociliary clearance has been shown in clinical pharmacologic studies. Enhancement of fluid secretion and mucociliary clearance facilitates expectoration and eases cough.

Pregnancy and Lactation

Preclinical studies as well as extensive clinical experience after the 28th week have shown no evidence of ill-effects during pregnancy. Nonetheless, the usual precautions regarding the use of drugs during pregnancy, especially during the first trimester, should be observed. The drug enters breast milk, but is not likely to affect the infant when therapeutic doses are used.

Side Effects

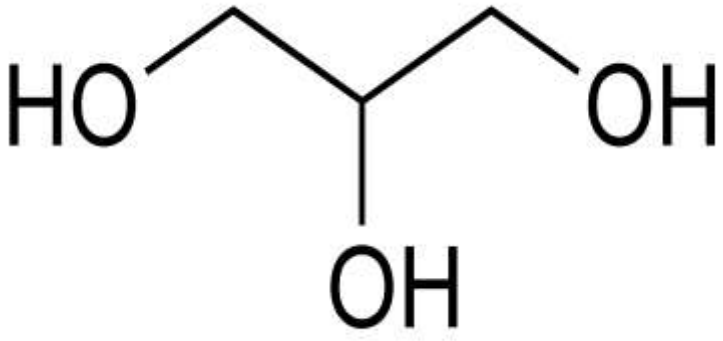
Mucosolvan is generally well tolerated. Mild upper gastro-intestinal side effects (primarily pyrosis, dyspepsia, and occasionally nausea, vomiting) have been reported, principally following parenteral administration. Allergic reactions have occurred rarely, primarily skin rashes. There have been extremely rare case reports of severe acute anaphylactic-type reactions but their relationship to ambroxol is uncertain. Some of these patients have also shown allergic reactions to other substances.

Overdosage

No symptoms of overdosage have been reported in man to date. If they occur, symptomatic treatment should be provided. Administration of ambroxol together with antibiotics (amoxicilline, cefuroxime, erythromycin, doxycycline) leads to higher antibiotic concentration in the lung tissue. No clinically relevant unfavorable interaction with other medications have been reported.

6. EXCIPIENT PROFILE [23]

Glycerin



Nonproprietary Names

- BP : Glycerol
- JP : Concentrated glycerin
- PhEur : Glycerolum
- USP : Glycerin

Synonyms : Croderol; E422; glycerine; Glycon G-100; Kemstrene; Optim; Pricerine; 1,2,3-propanetriol; trihydroxypropane glycerol.

Chemical Name : Propane-1,2,3-triol

Empirical Formula : $C_3H_8O_3$

Molecular Weight : 92.09

Functional Category : Antimicrobial preservative; emollient; Humectants; plasticizer; solvent; Sweetening agent; tonicity agent.

TYPICAL PROPERTIES

Boiling point : 290ºC (with decomposition)

Density : 1.2656 g/cm^3 at 15ºC

Melting point : 17.8ºC

SOLUBILITY TABLE :

Table no: 2 Solubility of glycerin.

Solvent	Solubility at 20ºC
Acetone	Slightly soluble
Benzene	Practically insoluble
Chloroform	Practically insoluble
Ethanol (95%)	Soluble
Ether	1 in 500
Ethyl acetate	1 in 11
Methanol	Soluble
Oils	Practically insoluble
Water	Soluble

VISCOSITY OF GLYCERIN:

Concentration of aqueous glycerin solution (% w/w)	**Viscosity at 208C (mPas)**
5	1.143
10	1.113
25	2.095
50	6.05
60	10.96
70	22.94
83	111.0

Table no: 3 viscosity of glycerin

SPECIFIC GRAVITY OF GLYCERIN:

Table no: 4 specific gravity of glycerin

Concentration of aqueous Gl ycerin solution (% w/w)	**Specific gravity at 20^0C**
10	1.024
20	1.049
30	1.075
40	1.101
50	1.128
60	1.156

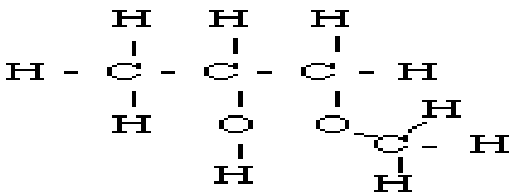
STABILITY & STORAGE

Condition : Glycerin is a stable hygroscopic material.The bulk material should be stored in a well closed container in cool and dry place.

Applications : In oral solutions, glycerin is used as a solvent, sweetening agent, antimicrobial preservative and viscosity-increasing agent. It is also used as a plasticizer and in film coatings Glycerin is additionally used in topical formulations such as creams and emulsions.

PROPYLENE GLYCOL

Structure formula



Nonproprietary names

- BP : Propylene glycol
- JP : Propylene glycol
- PhEur : Propylenglycolum
- USP : Propylene glycol

Synonyms : 1,2-Dihydroxy propane ; 2-hydroxy propanol; methyl glycol ;propane-1,2-diol

Chemical name : 1,2-propanediol.

Empirical Formula : $C_3H_8O_2$

Molecular weight : 76.09

Structural formula : $CH_3CHOHCH_2OH$

Functional Categor : Antimicrobial preservative, disinfectant, stabilizer for vitamins, humectants water miscible co-solvent, plasticizer.

Description : Clear, colorless, viscous, partially colorless liquid with a sweet, slightly acrid taste resembling glycerin.

TYPICAL PROPERTIES

Density : 1.038 g/cm3 at 50ºC

Boiling point : 188^0 C

Viscosity : 58.1 mPs at 50^0C

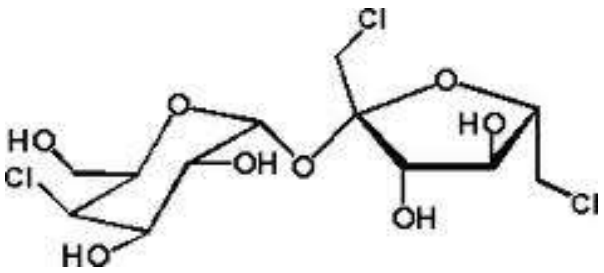
Solubility : Miscible with acetone, chloroform, ethanol (95%) glycerin, water. Soluble 1 in 6 parts of ether. Not miscible with light mineral oil, Dissolves in some essential oils.

Incompaillties : It is incompatible with oxidizing reagents such as potassium permanganate.

Applications : It is widely used as a solvent, extractant, preservative in a variety of parenteral and non parenteral preparation. Propylene glycol is commonly used as a plasticizer in aqueous film coating formulation.

SUCRALOSE

Structure formula



Nonproprietary names

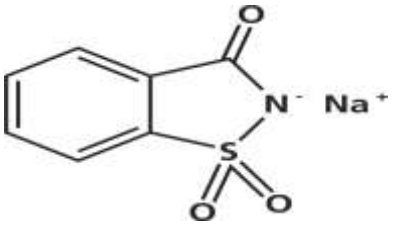
USPNF	**:** Sucralose
Synonyms	**:** 1,4,6-trichlorogalactosucrose; 4,1,6-trichloro-4,1,6-trideoxy-galacto-sucrose.
chemical name	**:** 1,6-Dichloro-1,6-dideoxy-β-D-fructofuranosyl-4-chloro-4-deoxy-α-H-galactopyranoside.
Empirical Formula	**:** $C_{12}H_{19}C_{l3}O_8$
Molecular weight	**:** 397.64
Functional Category	**:** Sweetening agent.
Description	**:** Sucralose is a white to off-white colored, free-flowing, Crystalline powder.

TYPICAL PROPERTIES

Density	: 0.35 g/cm3
Melting point	: 130^0C
Viscosity	: 0.6-3.8 mPas
Solubility	: Freely soluble in ethanol (95%), methanol, and water; slightly soluble in ethyl acetate.
Stability and Storage	**:** Sucralose should be stored in a well-closed container in a
Conditions	: cool and dry place.
Applications	: Sucralose is used as a sweetening agent in beverages, foods, and pharmaceutical applications. It has a sweetening power approximately 300–1000 times that of sucrose and has no aftertaste. It has no nutritional value.

SACCHARIN SODIUM

Structural formula



Nonproprietary names

- BP : Saccharin Sodium
- JP : Saccharin Sodium
- PhEur : Saccharinum natricum
- USPNF : Saccharin Sodium

Synonyms : 1,2-Benzisothiazolin-3-one 1,1-dioxide, Sodium salt; Crystalose; E954; sodium o-benzosulfimide; soluble gluside; soluble saccharin; sucaryl sodium.

Chemical Name : 1,2-Benzisothiazol-3(2H)-one1,1-dioxide, sodium salt.

Empirical Formula : $C_7H_4NNaO_3S$

Molecular weight : 205.16

Functional category : Sweetening agent.

Typical properties :

Density : 0.8-1.1 g/cm^3

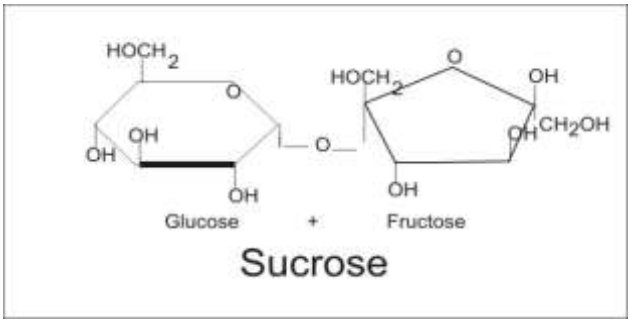
Melting point : Decomposes upon heating.

Stability & Storage Condition : Saccharin sodium is stable under the normal range of conditions employed in formulations. Saccharin sodium should be stored in a well closed container in a cool , dry place.

Applications : Saccharin sodium is an intense sweetining agent used in beverages, food products, table-top sweeteners, and pharmaceutical formulations such as tablets, powders, Medicated confectionery, gels, suspensions, liquids, and mouthwashes.

SUCROSE[23]

Structural formula :



Nonproprietary names

- BP : sucrose
- JP : sucrose
- PhEur : saccharum
- USPNF : sucrose

Synonyms : Beet sugar,cane sugar,-α-D-glucopyranosyl-β-D-fructofuranoside.

Chemical name : β-D-fructofuranosyl-α-D-glucopyranoside

Empirical formula : $C_{12}H_{22}O_{11.}$

Molecular weight : 342.30

Functional category : Coating agent, granulating agent, suspending agent, sweetening agent, tablet binder.

Description : Sucrose is a sugar obtained from sucrose occurs as colorless crystals or as a white crystalline powder. It is odorless and has a sweet taste.

Typical properties

Density : 0.93g/cm3

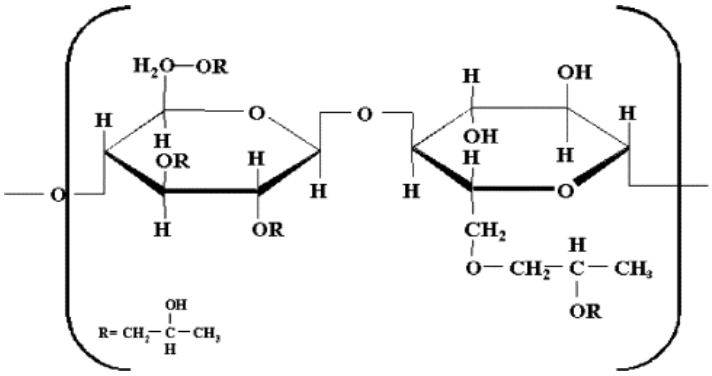
Melting point : 160-186^{o}c

Stability and storage conditions : Sucrose has good stability at room temperature. The bulk material should be stored in a well closed container in a cool,dry place.

Applications : Sucrose is widely used in oral pharmaceutical formulations. Sucrose syrup containing 50-60% - w/w sucrose, it is used in tableting as a binding agent for wet granulation.

HYDROXYPROPYL CELLULOSE

Structural formula



Nonproprietary names

- BP : Hydroxypropyl cellulose
- JP : Hydroxypropyl cellulose
- PhEur : Hydroxypropyl cellulosum
- USPNF : Hydroxypropyl cellulose

Synonyms : Cellulose, hydroxypropyl ether ; E463; Hyprolose; Klucel; Methocel;Nisso HPC; oxypropylated cellulose.

Chemical Name : Cellulose,2-hydroxypropyl ether

Molecular weight : 50,000-1,250,000

Functional category : coating agent; emulsifying agent; stabilizing agent; suspending agent; tablet binder; thickening agent; viscosity-increasing agent.

Typical properties

Density : 0.5 g/cm^3

Melting point : 260-275^0 C.

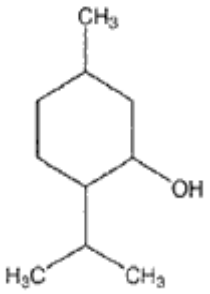
Stability & Storage Condition : Hydroxypropyl cellulose powder is a stable material, it was hygroscopic after drying. Hydroxypropyl cellulose powder Stored in a well closed container in a cool, dry place.

Incompatibilities : Hydroxypropyl cellulose in solution demonstrates such incompatibility with substituted phenol derivatives, Such as methylparaben and propylparaben. The presence of anionic polymers increase the viscosity hydroxypropyl cellulose solutions.

Applications : Concenration of 15-35% w/w of hydroxypropyl cellulose may be used to produce tablets with an extended drug release. Aqueous solutions containing hydroxypropyl cellulose along with an amount of methyl cellulose or ethanoic solutions may be used.

MENTHOL

Structural formula:



Nonproprietary names :

- BP : Racementhol
- JP : Menthol
- phEur : Mentholum racemicum
- Usp : Menthol

Synonyms : Hexahydrothymol,2-isopropyl-5- methycyclohexanol,4-iso-propyl-1-methylcyclohexan -3-ol;3-p-menthanol.

Chemical name : $C_{10}H_{20}O$

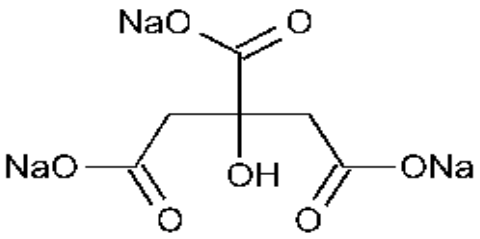
Molecular weight : 156.27

- **Specific gravity** : 0.890 at 15°C
- **Refractive index** : 1,458 at 25°C
- **Melting point** : l-Menthol 43-44°C; the USP allows a melting range of 41°-44°C racemic menthol displays a congealing point at two levels: 27-28°C and 30.5-32°C
- **Boiling point** : 212°C
- **Flash point** : 200°F/93°C
- **Solubility** : H_2O at 20°C = 0.04%; soluble in ethanol, essential oils, esters, alcohols, chlorinated solvents, mineral and edible oils etc.

Application : Menthol is widely used in pharmaceuticals confectionery, and toiletry products as a flavoring agent or odor enhancer. It is also used in orul syrups and inhalation,oral suspension,tablets and topical formulations.

SODIUM CITRATE DIHYDRATE[23]

Structure formula



Nonproprietary Names

- BP : sodium citrate
- Jp : sodium citrate
- Usp : sodium citrate
- Pheur : natrii citras

Synonyms : citric acid trisodium salt; SODIUM citrate tertiary , trisodium citrate.

Chemical name : Trisodium 2-Hydroxypropane-1,2,3- tricarboxyylate dehydrate.

Empirical formula : $C_6H_5NA_3O_7.2H_2O$

Molecular weight : 294.10

Functional category : Alkalizing agent, buffering agent, emulsifier, sequestering agent.

Description : sodium citrate dihydrate consists of odorless, colorless, a white crystalline powder.

Typical properties

Density : 1.12g/cm

Melting point : 150^{o} C

Stability and storage conditions : Sodium citrate dehydrate is a stable material. Aqueous solutions may be sterilized by autoclaving. On storage, aqueous solutions may cause the separation of small, solid particles from glass containers.

Incompatibilities : Aqueous solutions are slightly alkaline and will react with acidic substance. Alkaloidal salts may be precipitated from their aqueous or hydro-alcoholic solutions .Calcium and strontium salts will cause precipitation of the corresponding citrates. Other incompatibilities include bases, reducing agents, and oxidizing agents.

Application : It is used in food product. Adjust the pH of solution.it is also used as a sequestering agent.the anhydrous material is used in effervescent tablet formulations.

7. MATERIALS AND METHODS

7.1 Materials used in the study:

Table no. 5 List of materials used:

S.no :	Name of the materials	Manufacturer /supplier	Function
01	Ambroxol HCL	koraer inida-koraesina	Active ingredient
02	Sucrose	Dharani sugar pvt.ltd.T.N	Sweetner
03	Sodium saccharin	China pvt-ltd vardhaman & co.chennai	Sweetner
04	Sucralose	Tate&lyte Pvt-ltd KP manis global co.chennai	Sweetner
05	Propylene glycol I.P	Salicylate pvt-ltd chemicals Hyderabad	Preservative
06	Glycerin	Godroj industries Ltd. Mumbai	Viscosity builder
07	Hydroxypropyl cellulose	Laffano detro chemicals ltd. Gujarat.	Viscosity builder
08	Menthol	Hindustan Pvt-ltd vardhaman co Chennai.	Flavouring agent
09	Citric acid	Sunil chemical	Acidulate
10	Sodium citrate	Sunil chemical	Buffering agent
11	Flavouring oil	Aromatics India pvt ltd Daman.	Flavouring agent

7.2Equipments used in the study:

Table no: 6 List of instruments /equipments used:

s.no	Instrument /Equipments	Manufacture/supplier
01.	Electronic waghing balance	Shimadzu Japan.
02.	Viscometer	Lab equipement MMC pvt ltd. Chennai.
03.	pHmeter	Digisun electronics Hyderabad.
04.	Dissolution test apparatus	Veego (UDA-60)
05.	U.V Visible spectrophotometer	UV pharma spec 1700 Shimadzu
06.	HPLC	HPLC - LC 99 Shimadzu, Japan.
07.	TLC	Lab equipement MMC pvt ltd. Chennai.
08.	Water bath	Lab equipement MMC pvt ltd Chennai.
09.	Humidity chamber	Lab equipement MMC pvt ltd Chennai.
10	Hot air oven	Lab equipement MMC pvt ltd Chennai.
11	Melting point	Lab equipement MMC pvt ltd Chennai.
12	FTIR	Lab equipement MMC pvt ltd Chennai

7.3 PREFORMULATION STUDIES OF AMBROXOL HYDROCHLORIDE

It is one of the important prerequisite in development of any drug delivery system. Pre formulation studies were performed on the drug, which included melting point determination, solubility and compatibility studies.

7.3.1 Determination of melting point:[24]

Melting point of Ambroxol HCl was determined by capillary method. Fine powder of Ambroxol hydrochloride was filled in glass capillary tube (previously sealed on one end). The capillary tube is tied to thermometer and the thermometer was placed in flam. The powder at what temperature it will melt was noticed.

7.3.2 Solubility:

1mg Ambroxol HCl was dissolved in 100ml of different solvents like water methylene chloride, ethanol and ether separately and was tested for solubility as per BP 2000.

7.3.3 Determination of λ_{max}:

A solution of Ambroxol HCl containing the concentration 10 µg/ ml was prepared in 0.1N HCl and UV spectrum was taken using Shimadzu UV double beam spectrophotometer. The solution was scanned in the range of 200 to 400 nm.

7.3.4 Preparation of standard stock solution of ambroxol hydrochloride

30mg of ambroxol hydrochloride was dissolved in 100ml of 0.1N HCl. Aliquot of stock solution was further diluted by using the same solvent to obtain concentration range from 80mcg/ml- 120mcg/ml. The absorbance of the solutions was measured at 301nm using UV-visible spectrophotometer.

7.3.4.1 Preparation of 0.1N Hydrochloric acid

8.5ml of the hydrochloric acid was taken and dissolved in water and made upto 1000ml to get 0.1N hydrochloric acid.

Drug excipient compatibility study[25]

Compatibility studies were carried out to study the possible interactions between Ambroxol hydrochloride and other inactive ingredients. The compatibility studies were carried out with an aim to select a suitable excipient for a stable and best formulation. A blend of drug with the excipient in suitable ratios were filled in glass vials for exposing to 40^0C/75% RH and observed for physical changes.

Procedure:

APl and excipients were thoroughly mixed in predetermined ratio given in table below and the blend was filled in white colored glass vials and closed with gray rubber stoppers and sealed with aluminum seal and charged in to condition at 40^0 C/75 %RH. Similarly API was also kept per the sample.

Table no : 7 Compostion of drug and excipients used for Compatibility study

S.no	Composition details	Ratio
01	Drug	1
02	Drug +sodium saccharin	1:0.5
03	Drug+ menthol	1:0.1
04	Drug + hydroxypropyl cellulose	1:0.3
05	Drug + sucrose	1:2
06	Drug+ sucrolose	1:05

Chemical compatibility of the above samples was verified using FTIR spectrums.

Testing frequency:

1. Once a month for 3 months for sample charged at 40^0C/75%RH.

2. **7.3.6.1 Physical Observation:**

3. Physical observation of sample was done at every week for any colour change or lumps formation and flow.

4. Drug excipients compatibility studies:

7.3.5 Compatibility studies by FTIR:[26], [27]

Ambroxol HCl with polymers was subjected to IR studies Infrared (IR) spectroscopy was conducted using a FTIR Spectrophotometer and the spectrum was recorded in the wavelength region of 4000 to 400 cm^{-1}. The procedure consisted of dispersing a sample (drug alone and excipients) in KBr in the ratio of 100:1 and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained with principle peaks. The peaks obtained by IR spectroscopy are compared with drug standard IR spectra.

7.4 FORMULATION DEVELOPMENT

Following ingredients were selected to develop the desired formulation

Table no: 8 Composition used in the formulation of syrups:

Ingredients(g)	S-1	S-2	S-3	S-4	S-5
Ambroxol Hcl (g)	3	3	3	3	3
Propylene glycol(g)	50	100	150	50	50
Glycerin(g)	50	100	150	50	50
Sucralose(g)	1.5	1.5	1.5	-	-
Sodium saccharin(g)	-	-	-	1	-
Sucrose (g)	-	-	-	-	250
HPC (g)	1	1	1	1	1
Menthol (g)	0.5	0.5	0.5	0.5	0.5
Critic acid(g)	1	1	1	1	1
Sodium citrate(g)	1	1	1	1	1
DM Water qs	Make up 500 ml	Make up 500 ml	Make up 500 ml	Make up 500 ml	Make up 500 ml

Procedure:

Step: I

Propylene glycol was mixed with the active pharmaceutical ingredient Ambroxol hydrochloride.

Step: II

To the above mixture hydroxypropyl cellulose and glycerin were added and mixed well using 100 ml of water.

Step: III

The resultant mixture was stored in a well closed container for 20 hours or over night at room temperature.

Step: IV

0.5 ml of flavoring agent was then added and the pH was measured.

STEP: V

The pH was adjusted to pH 4.5by using citric acid solution and sodium citrate solution.

Step: VI

After 20 hrs or over night storage sucralose or sucrose or saccharin sodium was added and the volume was made upto 500 ml using distilled water.

7.5 EVALVATION OF SYRUP

7.5.1 pH [28]

pH is defined as the negative logarithm of hydrogen ion concentration.

Mathematically it is written as

$$pH = \log 1/[H_3o^+]$$

Since the logarithm of 1 is zero

The evolution may also be written

$$pH = - \log[H_3o^+]$$

Determination of pH

pH of syrup was determined by using pH meter. pH of the syrup also contributes to stability and characteristics of formulations. So pH of the syrup was recorded from time to time.

7.5.2 SPECIFIC GRAVITY[28]

The clean and dry empty specific bottle was weighed. Then the bottle was completely filled with distilled water and weighed. After cleaning and drying, the bottle was filled completely with the liquid whose specific gravity was to be determined and was weighed.

Observation:

Weight of empty dry specific gravity bottle = W_1g
Weight of specific gravity bottle filled with water =W_2g
Weight of specific gravity bottle filled with liquid =W_3g

Calculation :

Mass of water = $W_2 – W_1$ g
Mass of liquid = $W_3 - W_1$ g

Specific gravity = mass of liquid/mass of equal volume of water =W_3-W_1/W_2-W_1

7.5.3 VISCOSITY:[29]

Viscometer was thoroughly cleaned with a mixture of warm chromic acid. It was then filled with distilled water and clamped vertically onto a stand. The viscosity of the liquid to be determined is delivered from a pipette into the limb with bulb E. The quantity of liquid should

be such that, when it is sucked through the tube in the next limb, the upper level stands above the A mark and the lower level stands in the other limb at the bottom of bulb E. First, the distilled water was sucked until its upper meniscus in above A, its level marked and allowed to flow down. The stop clock was started when it reaches A mark and stopped when the level reaches B mark. The flow time was noted down in seconds in the tabular column. The procedure was repeated till the agreement values are obtained. The viscometer was cleaned again and equal volume of liquid was taken and the flow time was determined in second as above. The density of the liquid with specific gravity bottle was determined and viscosity was calculated

Time in seconds flow x viscometer factor x wt/ml of water x wt/ml of liquid.

7.6 MICICROBIAL EXAMINATION:[30]

PROCEDURE:

7.6.1 TOTAL BACTERIAL COUNT:

Preparation of media

Soyabean casein digest broth medium

Pancreatic digest of casein	17.0g
Papain digest of soya bean	3.0g
Sodium chloride	5.0g
Dipotassium hydrogen phosphate	2.5g
Dextrose monohydrate	2.5g
Water to	1000ml

Adjust the pH so that after sterilization it is 7.3±0.2.Sterilise.

Sample preparation:

1ml of sample was collected, using sterile pipettes. It was then transferred to 10ml of soyabean casein digest broth medium.

Procedure:

1ml of the sample was pipetted out into two separate sterilized petridishes.15ml of the soyabean casein digest agar medium (SCDA) in petridish, was added at not more than 45^{o}C. Sample was mixed with media by slightly shaking the plates for uniform mixing of the sample and allowed to solidify. Incubate at 30-35^{o}C for 3 days. A negative control is also prepared in the same manner for comparison.

7.6.2 TOTAL FUNGAL COUNT:

Preparation of media

Sabouraud chloramphenicol agar medium (SCA)

Peptones (meat and casein)	10.0g
Dextrose monohydrate	40.0g
Agar	15.0g
Water to	1000 ml

Adjust the pH so that after sterilisation it is 5.6 ± 0.2 sterilise. Immediately before use, add 0.1 g of benzylpenicillin sodium and 0.1 g of tetracycline or alternatively add 50 mg of chloramphenical per litre of medium as sterile solution.

Sample Preparation:

1ml of the sample was collected**,** using sterile pipettes. It was transferred to 10ml of Sabouraud chloramphenicol agar medium (SCA).

Procedure:

1ml of the sample was pipetted out into two separate sterilized petridishes, 15ml of the Sabouraud chloramphenicol agar medium (SCA) in petridish was poured, at not more than 45˚C. Sample was mixed with media by slightly shaking the plates for uniform mixing of the sample and allowed to solidify. It was then incubated at 20-25˚C for 5 days. A negative control is also prepared in the same manner for comparison.

INTERPRETATION OF RESULTS:

For Total bacterial count:

CFU /ml = Average CFU obtained on SCDA plates x 10
(10 is dilution factor)
If no microbial colonies observed from the incubated petriplates express the results as < 10 CFU /g of sample.

For Total Fungal count:

CFU / ml = Average CFU obtained on SCA plates x 10
(10 is dilution factor)
If no microbial colonies observed from the incubated petriplates express the results as < 10 CFU /g of sample.

Acceptance criteria:

Total Bacterial count:	100 CFU/ml
Total Fungal count:	10 CFU /ml

7.6.3 TEST FOR PATHOGENS

7.6.3.1 For E.coli:

Preparation of media:

Mac-Conkey broth

Pancreatic digest of gelatin	20.0 g
Lactose	10.0 g
Dehydrated ox bile	5.0 g
Bromocersol purple	10 mg
Water to	1000 ml

Adjust the pH so that after sterilization it is 7.3 ± 0.2. Sterilize.

Procedure:

1ml of the above prepared sample was transferred to 100ml of Mac-Conkey broth and incubated at 30-35˚C for 24-48 hrs.

7.6.3.2 For Salmonella:

Preparation of media

Soyabean casein digest broth medium

Pancreatic digest of casein	17.0g
Papain digest of soya bean	3.0g
Sodium chloride	5.0g
Dipotassium hydrogen phosphate	2.5g
Dextrose monohydrate	2.5g
Water to	1000ml

Adjust the pH so that after sterilization it is 7.3±0.2.Sterilise.

Rappaport Vassiliadis Salmonella enrichment broth

Soya peptone	4.5g
Sodium chloride	7.2g
Potassium dihydrogen phosphate	1.26g
Di-potassium hydrogen phosphate	0.18g
Magnesium chloride (anhydrous)	13.58
Malachite green	0.036g

Adjust the pH to 5.2±0.2 at 25ºC

Directions:

Suspended 26.75g in 1 litre of distilled water and heated gently to diisolve. 10ml of the above was dispensed into screw-capped bottles or tubes and sterilised by autoclaving at 115ºC for 15 min.

Xylose- Lysine- Desoxycholate agar

Xylose	3.5g
L-Lysine	5.0g
Lactose	7.5g
Sucrose	7.5g
Sodium chloride	5.0g
Yeast extract	3.0g
Phenol red	80mg
Agar	13.5g
Sodium desoxycholate	2.5g
Sodium thiosulphate	6.8g
Ferric ammonium citrate	800mg
Water to	1000ml

Directions:

Adjusted the pH to 7.4±0.2. heated just to boiling and cooled to 50ºC and poured into petri dishes.

Sample preparation:

10 ml of the sample was transferred to 100 ml of soyabean casein digest broth and incubated at 30-35^0 C for 18-24 hrs. From this 0.1ml of the sample was tranferred to a test tube containing Rappaport Vassiliadis Salmonella enrichment broth and incubated at 30-35ºC for 24-48hrs. It was then subcultured on a plate of xylose lysine deoxycholate agar and incubated at 30-35ºC for 24-48 hrs.

7.6.3.3 For Pseudomonas:

Preparation of media:

Cetrimide agar media:

Pancreatic digest of gelatin	20g
Magnesium chloride	1.4g
Potassium sulphate	10g
Cetrimide	0.3g
Agar	13.6g
Glycerin	10.0g
Water to	1000ml

Directions:

Heated for 1min with shaking and the pH was adjusted to 7-7.4 and sterilized.

Sample preparation:

The prepared sample was subcultered on a plate of cetrimide agar and incubated at 30-35ºC for 18-72hrs.

7.6.3.4 For Staphylococcus:

Mannitol salt agar medium:

Pancreatic digest of casein	5.0g
Peptic digest of animal tissue	5.0g
Beef extract	1.0g
D-Mannitol	10.0g
Sodium chloride	75.0g
Agar	15.0g
Phenol red	25mg
Water to	1000ml

Directions:

The above mixture was mixed well and heated with frequent agitation and boiled for 1 min to effect the solution. After sterilization the pH was adjusted to 7.4±0.2.

Sample preparation:

The sample preparation was subcultured on a plate of Mannitol salt agar (MSA) and incubated at 30-35ºC for 18-72hrs

Acceptance criteria: Pathogens:Should be absent

.**ASSAY**

Accurately measured 10 ml of syrup was transferred to a 100 ml volumetric flask. Volume was made up with 0.1 N HCl. From the above 5ml of the solution was withdrawn and made upto 50ml with 0.1 N HCl and sonicated for 30 min .The absorbance of the resulting solution was measured at 308 nm.

7.7 RELATED SUBSTANCES

Determination by liquid chromatography.

Test solution:

Dissolve 50 mg of the substance under examination in water and dilute to 50 ml with the same solvent.

Reference solution (a).

Dissolve 5 mg of ambroxol hydrochloride RS in 250 ml of water. Dilute 5 ml of the solution to 100 ml with the mobile phase.

Reference solution (b).

Dissolve 5 mg the substance under examination in 0.2 ml of methanol and add 0.04 ml of a mixture of 1 volume of formaldehyde solution and 99 volumes of water. Heat at 60^0C for 5minutes. Evaporate to dryness under a current of nitrogen .Dissolve the residue in 5 ml of water and dilute to 20 ml with the mobile phase.

Chromatographic system.

A stainless steel column 25 cm x 4mm, packed with octadecylsilane bonded to porous silica.

Mobile phase : a mixture of equal volumes of acetonitrile and a buffer solution prepared by dissolving 1.32 g of ammonium phosphate in 9000 ml of water , adjusting the pH to 7.0 with phosphoric acid and diluting to 1000 ml with water

Flow rate .1 ml per minute

Spectrophotometer set: at 254 nm,

A 20µl loop injector.

Inject reference solution (b).The test is not valid unless the resolution between the secondary peak (trans-4-6,8-dibromo-1,4-dihydroquinazolin-3(2H)-yl)cyclohexanol) and the Ambroxol peak is at least 4.0.

Inject the test solution and reference solution (a). Continue the chromatography for 3 times the retention time of the principal peak in the chromatogram obtained with the test solution. The area of any secondary peak in the chromatogram obtained with the test solution is not more than 5 times the area of the principal peak in the chromatogram obtained with reference solution(a)(0.5 percent).The sum of the areas of all the secondary peaks is not more than 10 times the area of the principal peak in the chromatogram obtained with reference solution (a)(1.0 percent).Ignore any peak with an area 0.1 times the area of the principal peak in the chromatogram obtained with reference solution (a)(0.01 percent).

7.8 DISSOLUTION STUDY:[31]

5ml of Ambroxol HCl syrup was taken and the *in-vitro* drug release was studied using USP (type II) paddle apparatus with a speed at 50rmp. Dissolution was tested in acidic medium (0.1N HCl) of 900 ml at 37± 0.5^0C. Samples were withdrawn at 15, 30, 45 and 60min and filtered through 0.45 µ membrane filter and the absorbance of the resulting solution was measured at 308 nm using U.V visible spectrophotometer after suitable dilution.

7.9 STABILITY TESTING

STABILITY STUDIES OF THE FINISHED PRODUCT.[32]

Stability of a drug can be defined as the time from date of manufacture and packaging of the formulation until its chemical or biological activity is not less than a predetermined level of labeled potency and its physical characteristics have not changed appreciably or deleteriously. Although there are exceptions 90% of labeled potency is recognized as the minimum acceptable potency level.

The international conference on harmonization (ICH) guideline titled. "Stability testing of new drug substance and products (QIA)" describes the stability test requirements.

ICH specifies the length of study and storage conduction.

- Accelerated testing 40^0C± 2^0C/75% RH±5% and 30 ± 2°C/65±5%RH for 6months.

  Stability studies for the present work was carried out at 40^0 C± 2^0C 175% RH for the selected formulation for three months.

Method:

The selected formulations were packed in wide mouthed bottle. They were stored at 40º±2ºC/75±5%RH for 3 months in humidity chamber and evaluated for their physical appearance and various parameters at specified intervals of time.

8. RESULTS AND DISCUSSION

8.1 Preformulation study of Ambroxol HCl

8.1.1 Melting point determination:

Melting point of Ambroxol HCl was found to be 237°C.

8.1.2 Solubility:

Table No: 9 Solubility of Ambroxol HCl in various solvents

Sr. No.	Solvents	Solubility
1	Water	Sparingly soluble
2	Methylene chloride	Slightly soluble
4	Ethanol	Soluble
6	Ether	Practically insoluble

8.1.3 Determination of λ_{max}:

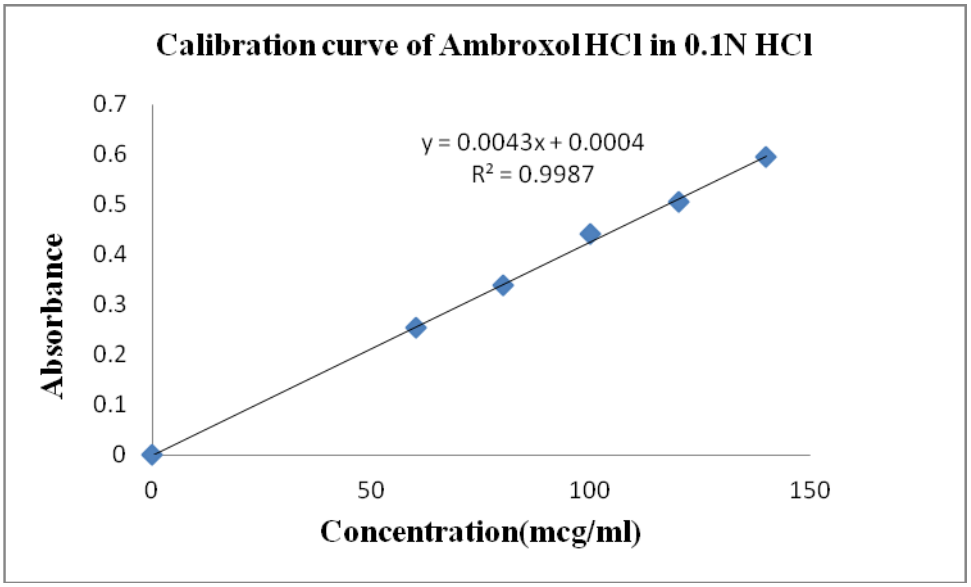
From the UV spectrum of drug, it was concluded that the drug had λ_{max} of 308 nm

8.1.4 Standard calibration curve of Ambroxol HCL :

Table No: 10 Absorbance data for the calibration curve of Ambroxol HCl in 0.1N HCl

S. No	Concentration (µg/ml)	Absorbance
1	0	0
2	60	0.254
3	80	0.338
4	100	0.422
5	120	0.505
6	140	0.596

Fig No: 1 Standard calibration curve of Ambroxol HCL in 0.1N HCl



8.2 Drug excipient compatibility study

Table no: 11 Physical incompatibility study of API and excipients

S.no	Composition details	Ratio	STROAGE 40^0C/75%RH. 30^{th} day
01	Drug	1	NCC
02	Drug +sodium saccharin	1:0.5	NCC
03	Drug+ sucrose	1:0.1	NCC
04	Drug+sucrolose	1:0.3	NCC
05	Drug + hydroxypropyl cellulose	1:2	NCC
06	Drug + menthol	1:05	NCC

8.1.5 Compatibility studies by FTIR:

Fig No: 2 FT-IR of Ambroxol HCl

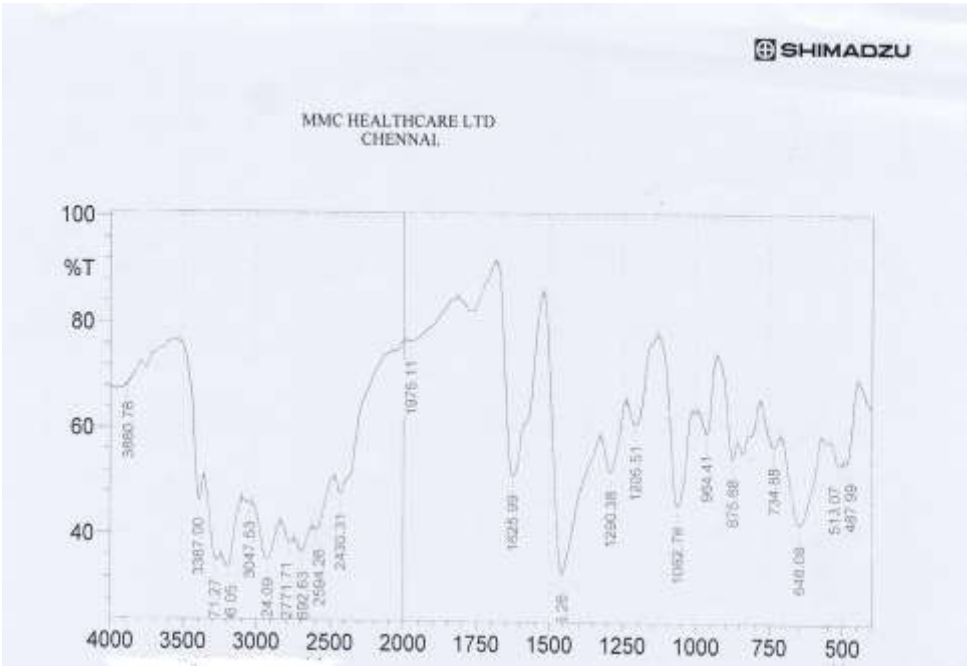


Fig No: 3 FT-IR of Ambroxol HCl and Sodium saccharin

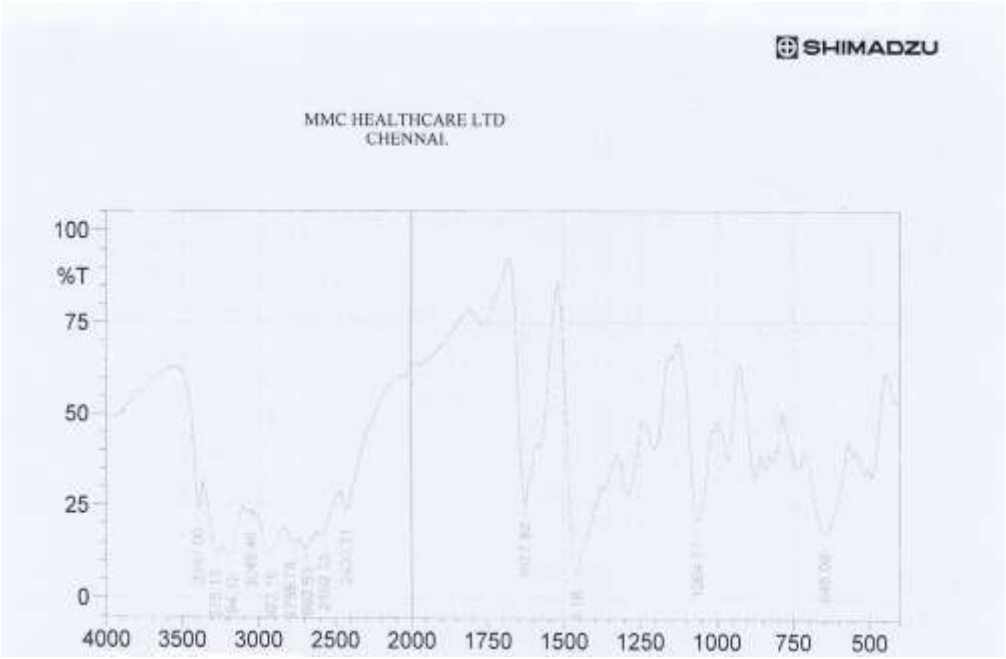


Fig No: 4 FT-IR of Ambroxol HCl and Menthol

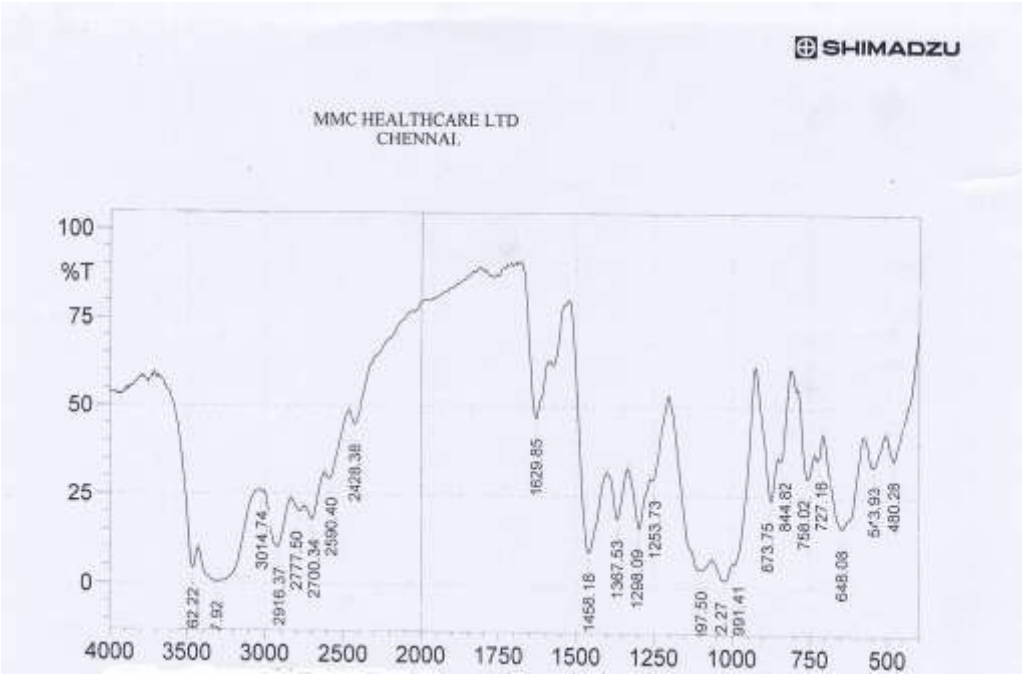


Fig No: 5 FT-IR of Ambroxol HCl and Hydroxypropyl cellulose

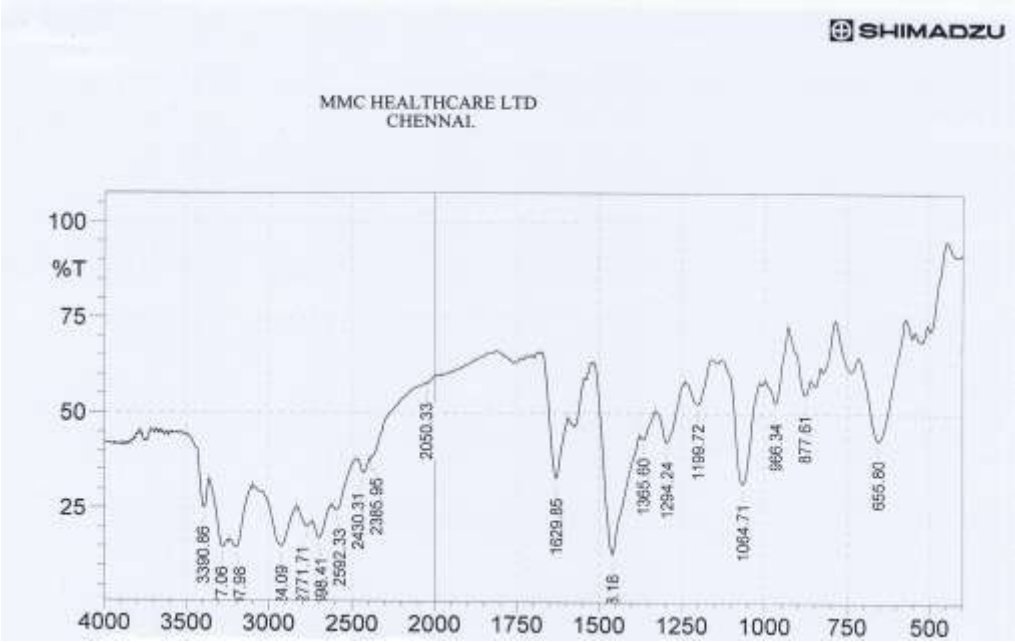


Fig No: 6 FT-IR of Ambroxol HCl and Sucrose

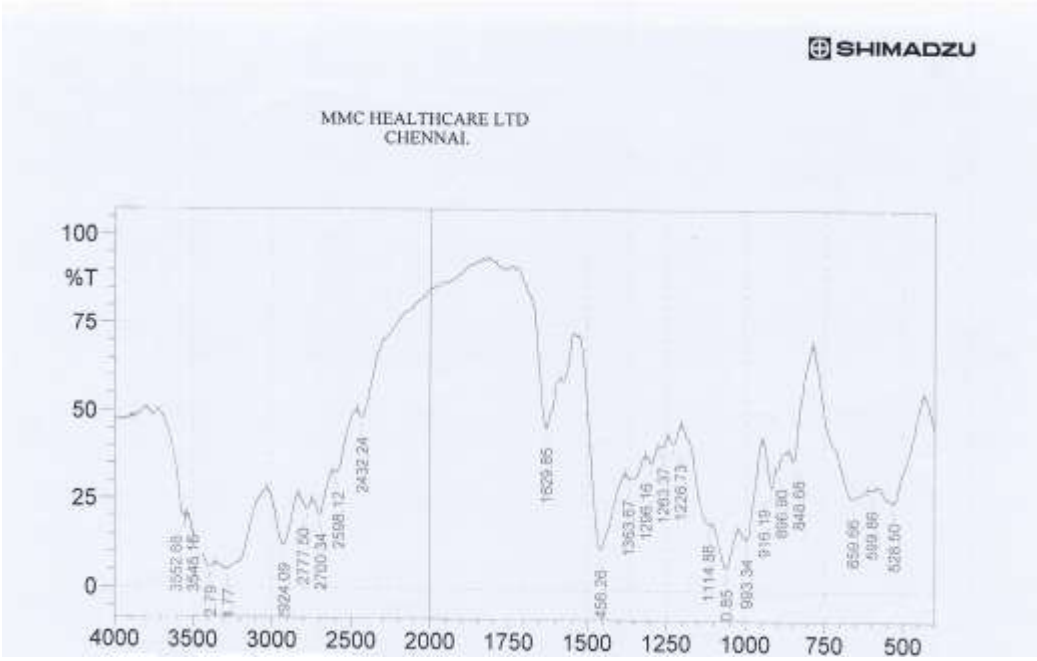


Fig No: 7 FT-IR of Ambroxol HCl and Sucrolose

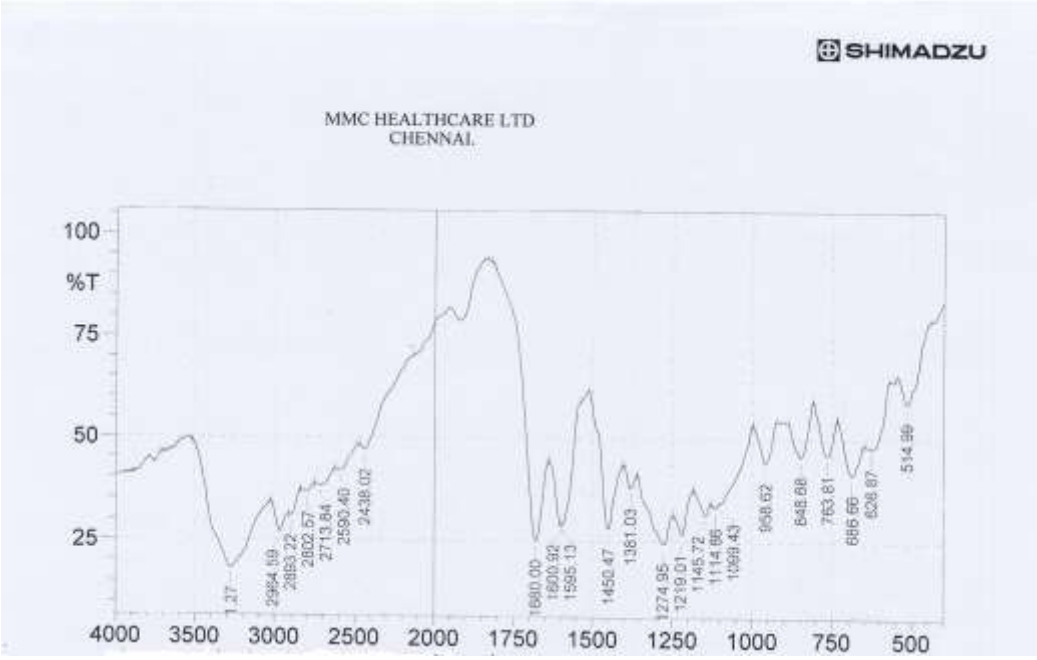


Table No: 12 FT-IR vibrations

S. No.	Type of Vibrations	Range
1	$-NH_2$	1627
2	O – H	3387
3	C – Br	500-650

The IR spectrum of Ambroxol HCL , Ambroxol HCL and excipient were interpreted and identified. The chief absorption bands of the drug are present in the Ambroxol HCL excipients mixture with same degree of sharpness and position it indicates that there is an absence of physical and chemical interactions among both active component and the excipient.

8.3 Study of different parameters of formulations

Table no: 13 Study of various parameters of different formulations

Batches	pH	Specific gravity	Viscosity	Assay	Total Viable Count
S1	4.51	1.12	5.09	109.74	10
S2	4.65	1.16	15.52	100.13	10
S3	4.72	1.18	30.44	102.97	10
S4	4.55	1.88	8.86	96.61	10
S5	4.57	1.18	26.98	99.12	10

- Pathogens, yeast and mould tests were completely negative.

7.7 RELATED SUBSTANCES

Fig No: 8 HPLC of Test solution-Trial1

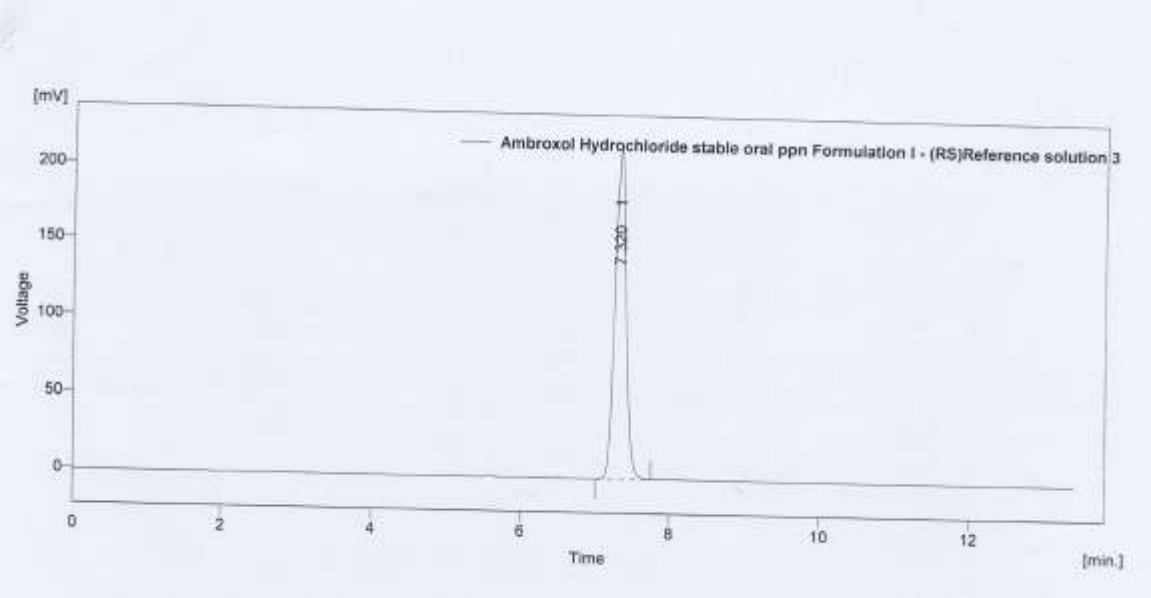


Fig No: 9 HPLC of Test solution-Trial 2

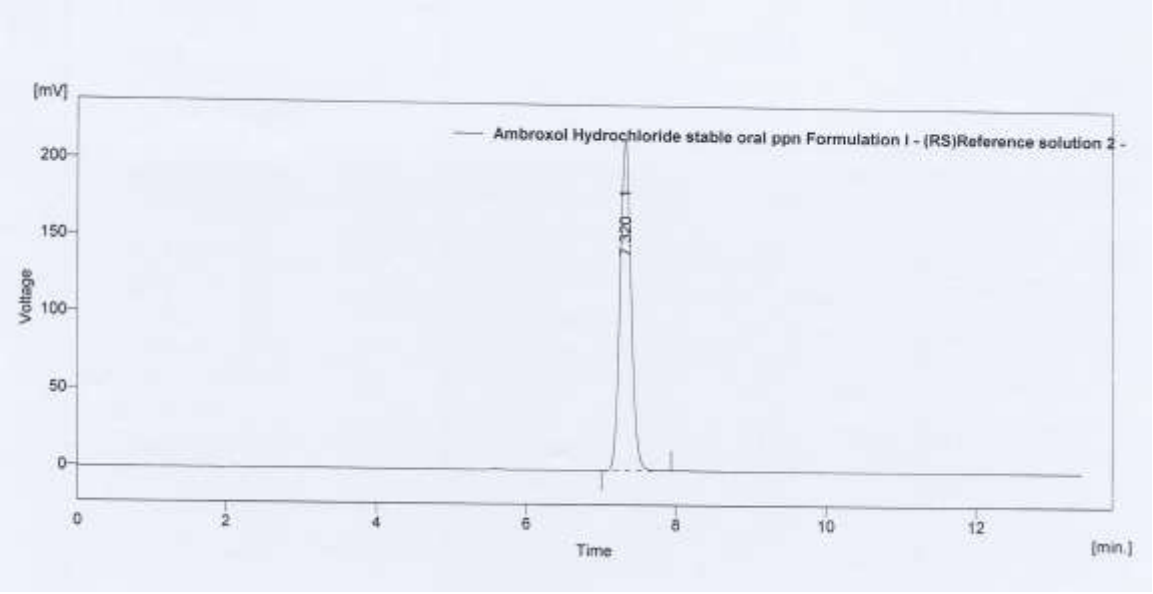


Fig No: 10 HPLC of Test Solution-Trial 3

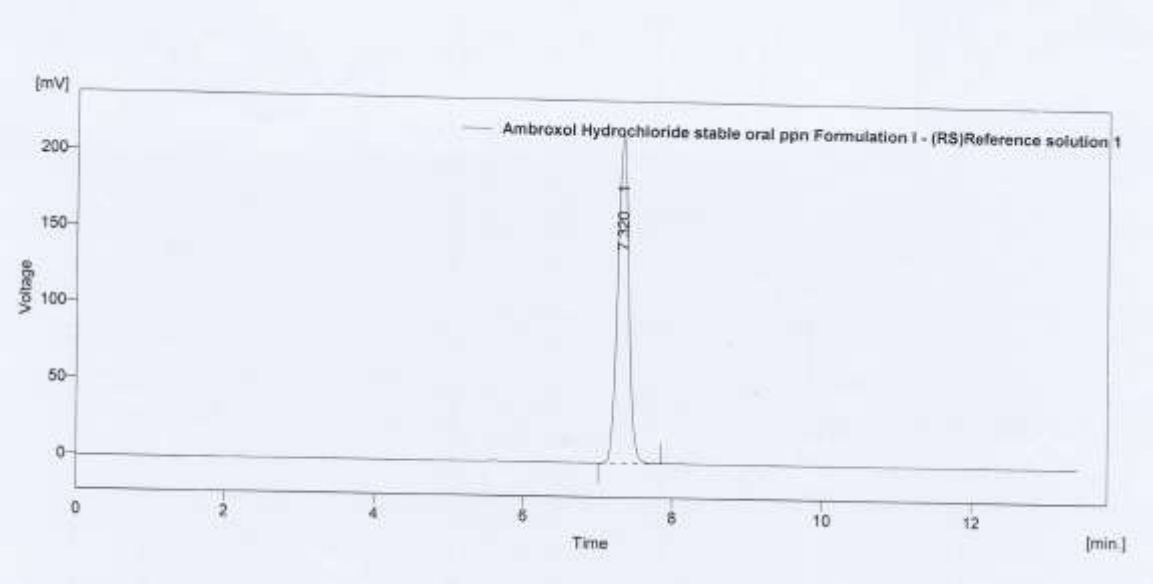


Fig No: 11 HPLC of S1 formulation

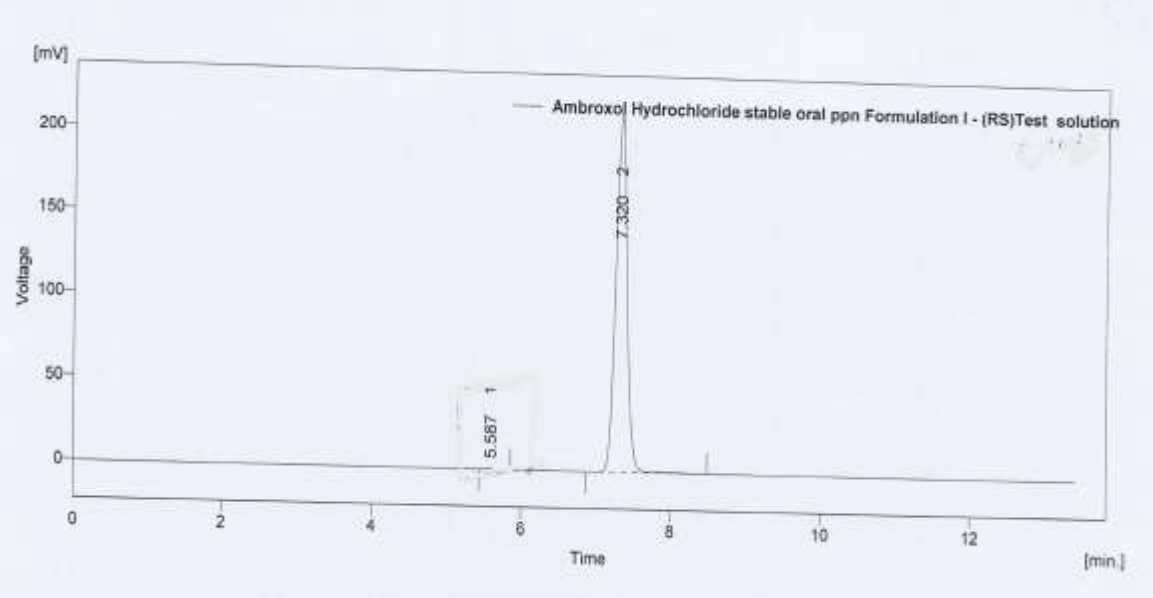


Fig No: 12 HPLC of S2 formulation

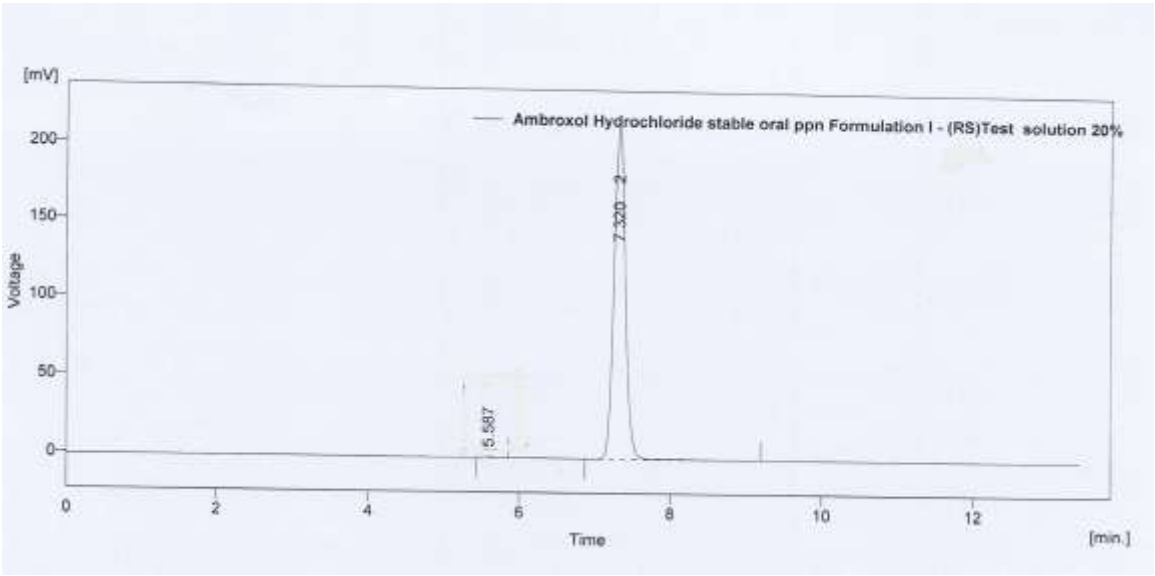


Fig No: 13 HPLC of S3 formulation

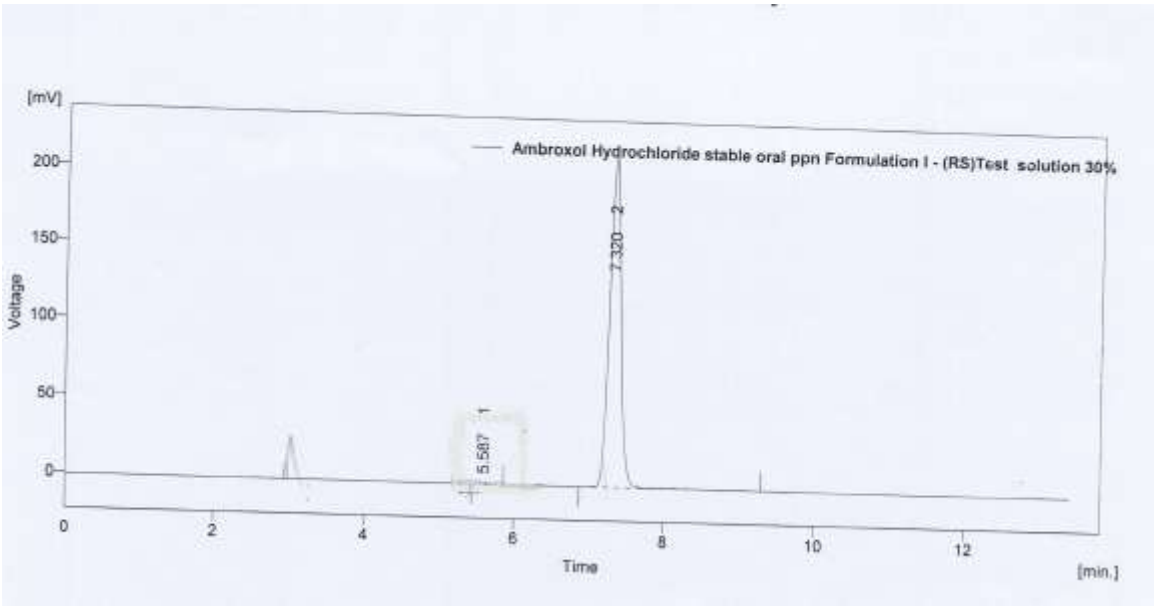


Fig No: 14 HPLC of S4 formulation

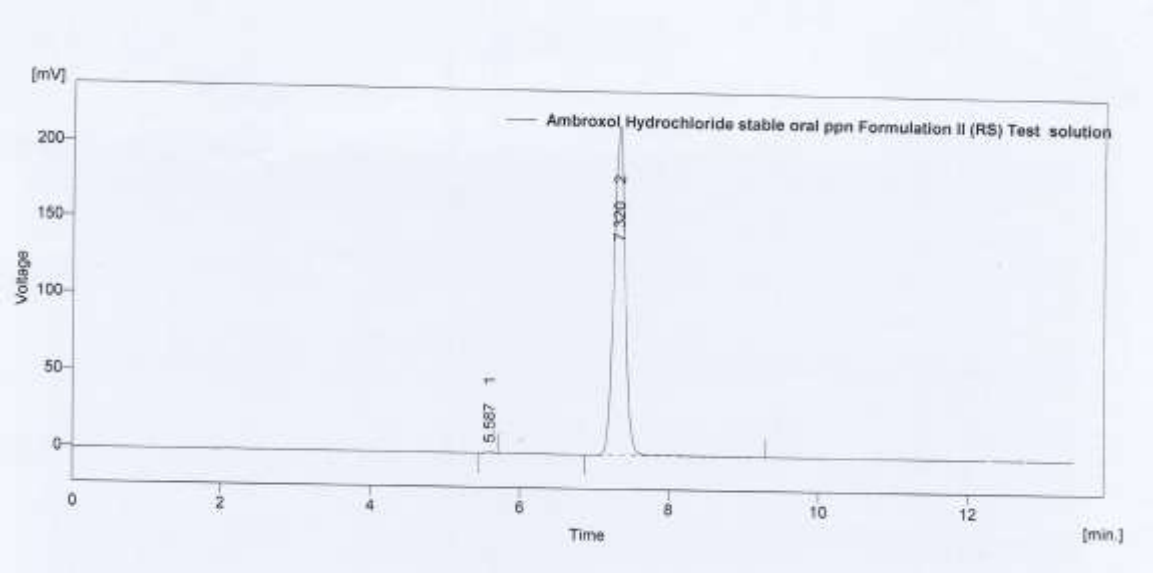
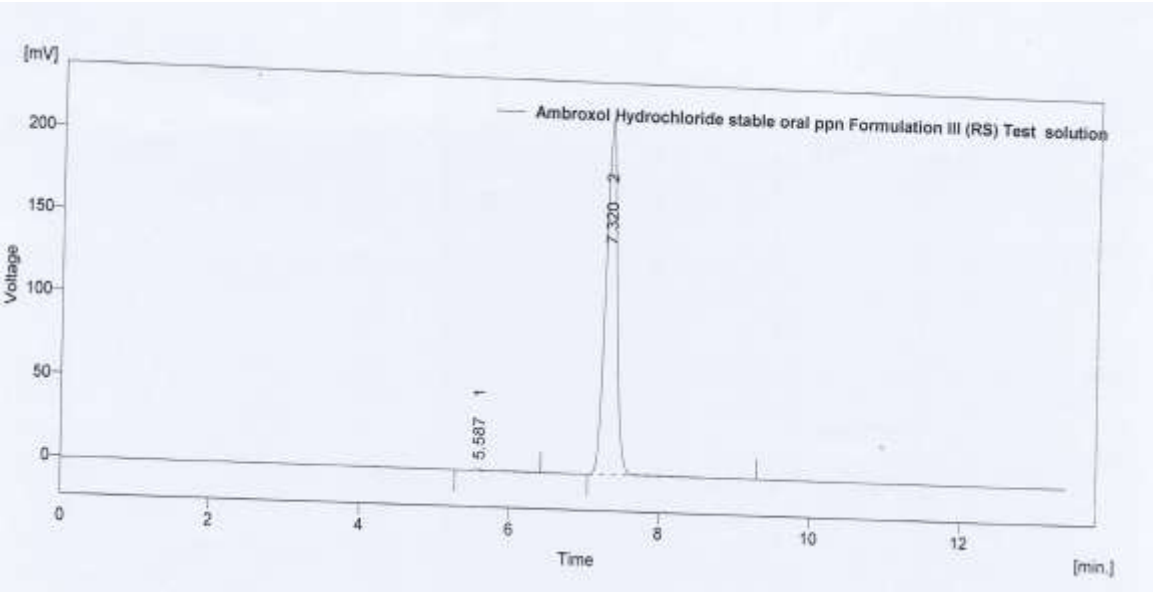


Fig No: 15 HPLC of S5 formulation



7.8 DISSOLUTION STUDY

Table No: 14 *In-vitro* drug release data for all formulations and marketed product

SI .NO.	TIME (MIN)	CUMULATIVE % DRUG RELEASE						
		S1	S2	S3	S4	S5	MARKET SAMPLE (SYRUP)	MARKET SAMPLE (TABLET)
1	15	105.03	86.32	84.07	86.32	86.04	84.07	58.11
2	30	107.67	87.84	84.54	87.84	86.06	86.04	68.51
3	45	111.37	89.36	88.04	88.04	88.37	86.06	80.08
4	60	113.48	91.87	88.37	89.36	88.40	88.40	86.54

Fig No: 16 *In-vitro* drug release profile for S1 formulation

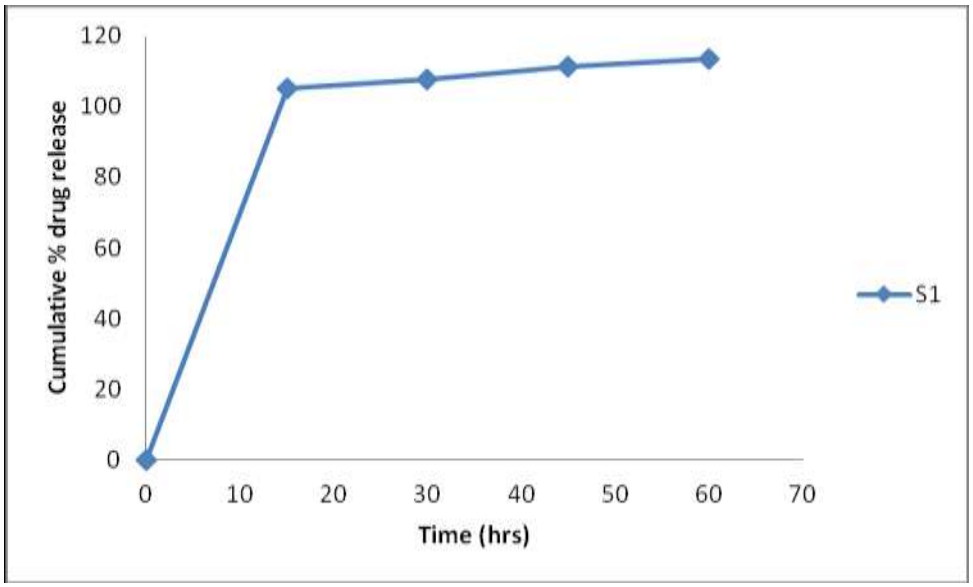


Fig No: 17 *In-vitro* drug release profile for S2 formulation

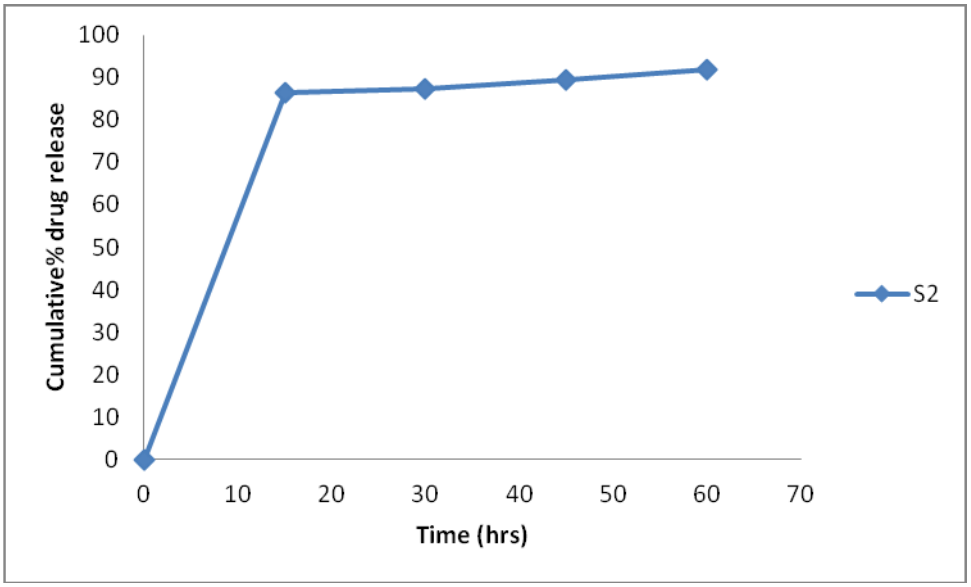


Fig No: 18 *In-vitro* drug release profile for S3 formulation

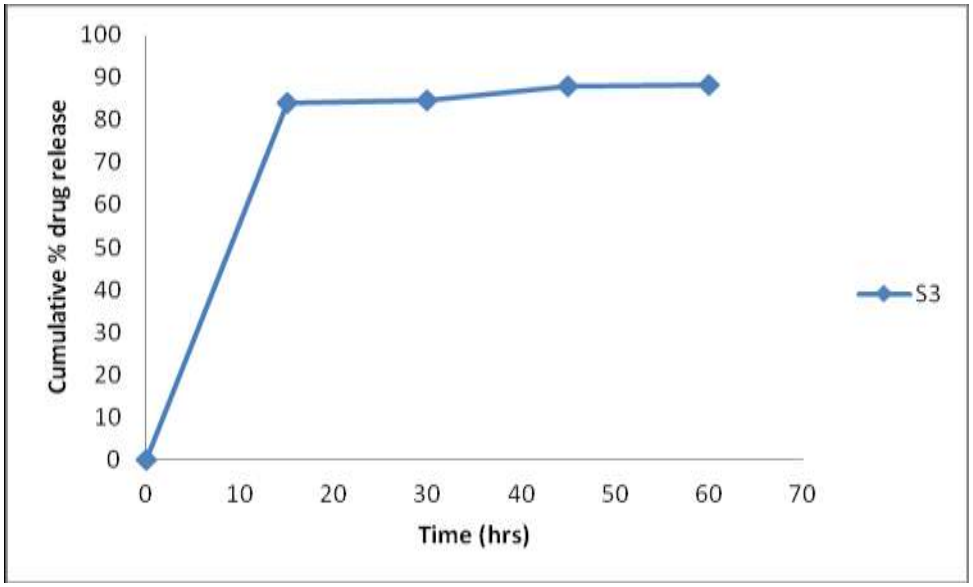


Fig No: 19 *In-vitro* drug release profile for S4 formulation

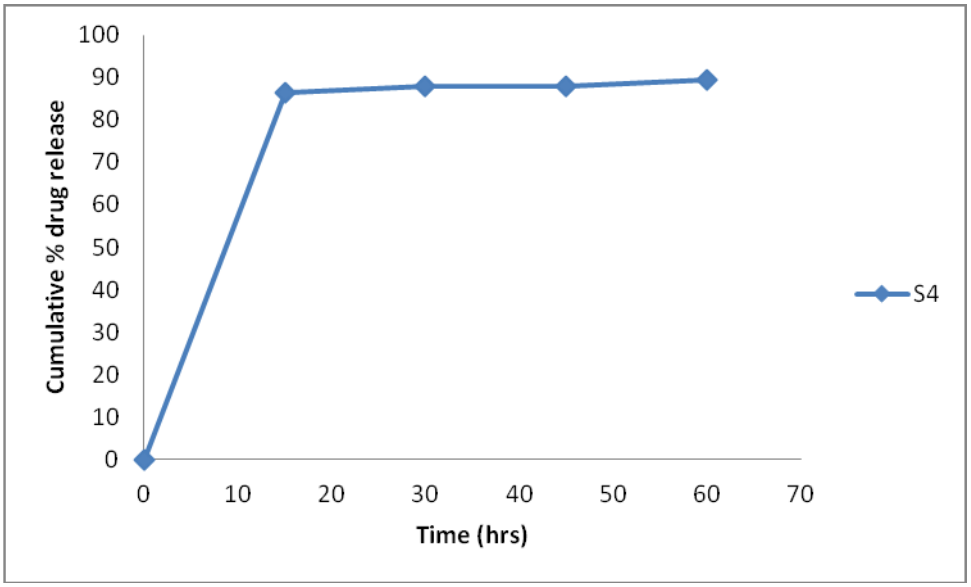


Fig No: 20 *In-vitro* drug release profile for S5 formulation

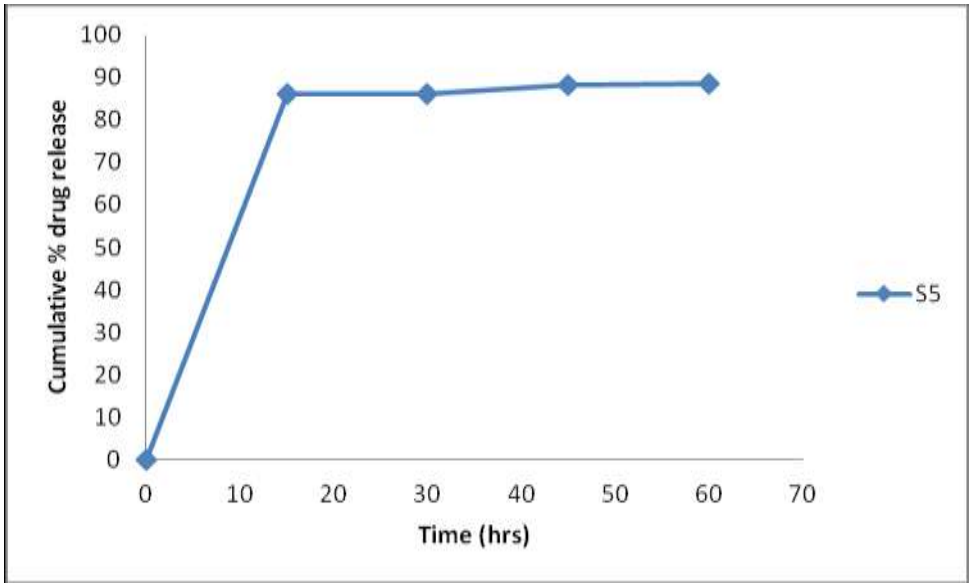
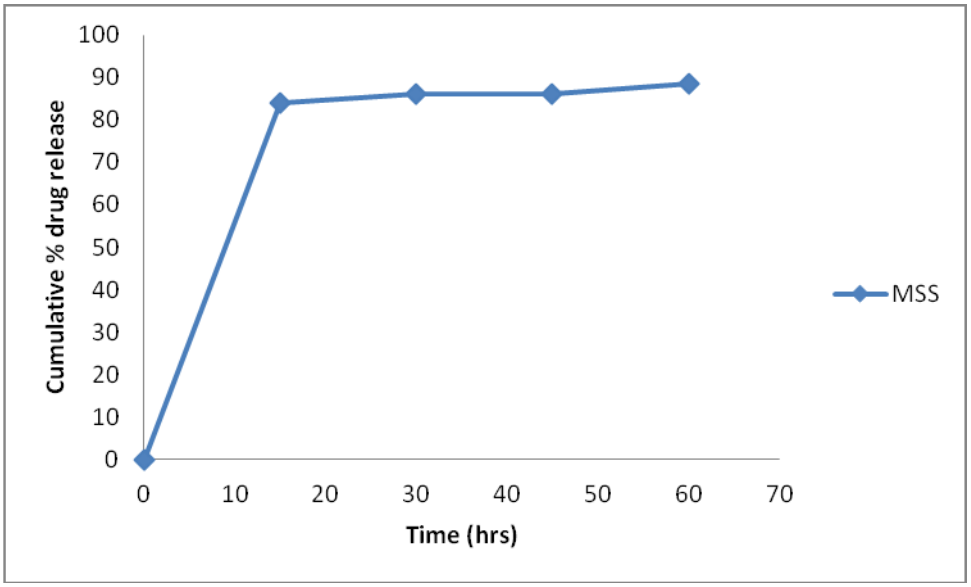


Fig No: 21 *In-vitro* drug release profile for MSS formulation



SIMILARITY FACTOR:

Comparison of the S1-S5 formulations with the innovator product was done by using the formulae given below:

$$f_2 = 50\text{Log}\ \{[1+1/n\ \text{Wt}\ \Sigma_n^{t=1}\ (\text{Rt-Tt})\ 2]\ -0.5\ \times 100\}$$

Table No: 15 Similarity factor all formulations with MSS

SI.NO	Comparison	f_2 value
1	MSS+S1	31.5
2	MSS+S2	76.82
3	MSS+S3	92.24
4	MSS+S4	84.2
5	MSS+S5	87.02

Fig No: 22 Comparison of *In-vitro* release profile of S3 and MSS

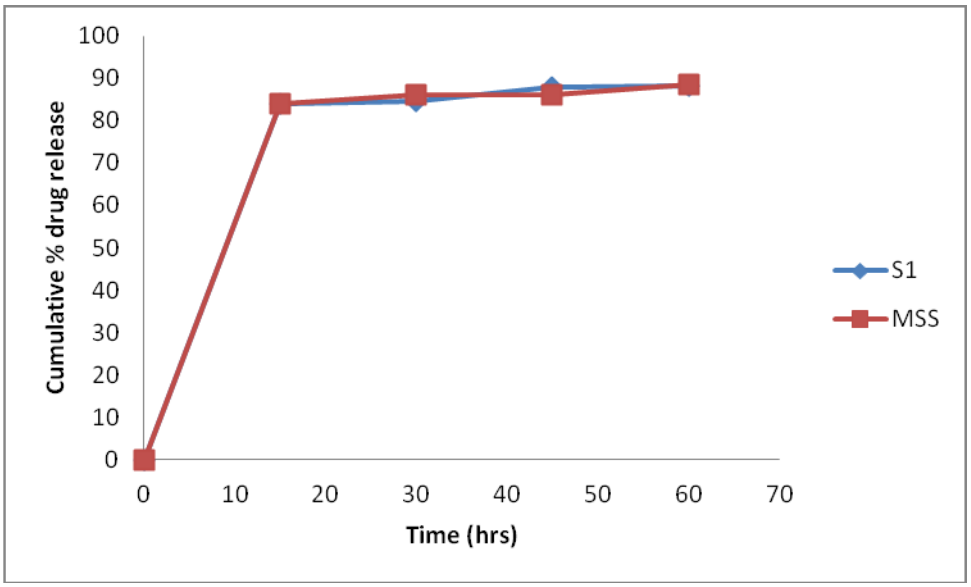
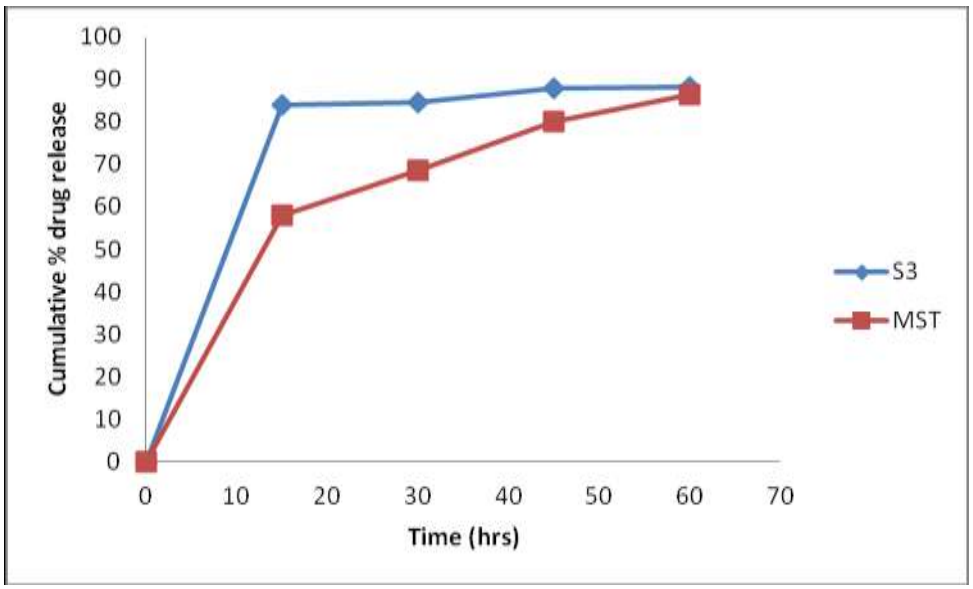


Fig No: 23 Comparison of *In-vitro* release profile of S3 and MST



7.9 **STABILITY TESTING**

Table No: 16 Stability studies of S1 formulation

TEST Specification		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Description	Colourless flavoured clear syrupy liquid	NCC	NCC	NCC	NCC
Identification	Positive for the Ambroxol hydrochloride	NCC	NCC	NCC	NCC
Recoverable Volume	60ml	60ml	60ml	60ml	60ml
pH	Lt: 4.0-4-5.0	4.51	4.67	4.72	4.58
Specific gravity	Lt:1.10gm/ml-1.25 gm/ml	1.12	1.15	1.16	1.12
Viscosity	Lt:5mPas to 8mPas	5.99	7.36	7.38	7.31
Assay	Lt:90% - 110%	109.74	106.72	106.63	105.49

Table No: 17 Stability studies of S2 formulation

TEST Specification		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Description	Colourless flavoured clear syrupy liquid	NCC	NCC	NCC	NCC
Identification	Positive for the Ambroxol hydrochloride	NCC	NCC	NCC	NCC
Recoverable Volume	60ml	60ml	60ml	60ml	60ml
P^H	Lt: 4.0-4-5.0	4.65	4.72	4.59	4.78
Specific gravity	Lt:1.10gm/ml-1.25 gm/ml	1.16	1.16	1.13	1.17
Viscosity	Lt:15mPas to 18mPas	15.52	15.95	15.23	16.10
Assay	Lt:90% - 110%	100.13	97.12	97.78	97.09

Table No: 18 Stability studies of S3 formulation

TEST Specification		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Description	Colourless flavoured clear syrupy liquid	NCC	NCC	NCC	NCC
Identification	Positive for the Ambroxol Hcl	NCC	NCC	NCC	NCC
Recoverable Volume	60ml	60ml	60ml	60ml	60ml
P^H	Lt: 4.0-4-5.0	4.72	4.62	4.78	4.58
Specific gravity	Lt:1.10gm/ml-1.25 gm/ml	1.18	1.20	1.17	1.19
Viscosity	Lt:30mPas to 33mPas	30.44	31.84	31.97	32.01
Assay	Lt:90% - 110%	102.97	97.12	97.57	96.38

Table No: 19 Stability studies of S4 formulation

TEST Specification		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Description	Colourless flavoured clear syrupy liquid	NCC	NCC	NCC	NCC
Identification	Positive for the Ambroxol Hcl	NCC	NCC	NCC	NCC
Recoverable Volume	60ml	60ml	60ml	60ml	60ml
P^H	Lt: 4.0-4-5.0	4.55	4.78	4.82	4.76
Specific gravity	Lt:1.10gm/ml-1.25 gm/ml	1.18	1.20	1.16	1.17
Viscosity	Lt:8mPas to 10mPas	8.86	9.45	9.59	9.43
Assay	Lt:90% - 110%	96.61	93.22	90.11	87.73

Table No:20 Stability studies of S5 formulation

TEST Specification		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Description	Colourless flavoured clear syrupy liquid	NCC	NCC	NCC	NCC
Identification	Positive for the Ambroxol Hcl	NCC	NCC	NCC	NCC
Recoverable Volume	60ml	60ml	60ml	60ml	60ml
P^H	Lt: 4.0-4-5.0	4.57	4.76	4.83	4.78
Specific gravity	Lt:1.10gm/ml-1.25 gm/ml	1.18	1.12	1.16	1.15
Viscosity	Lt:26mPas to 28mPas	26.98	26.82	27.40	27.46
Assay	Lt:90% - 110%	99.12	96.68	96.40	95.68

Fig No: 24 HPLC of Test solution-Trial 1 after 1month of stability

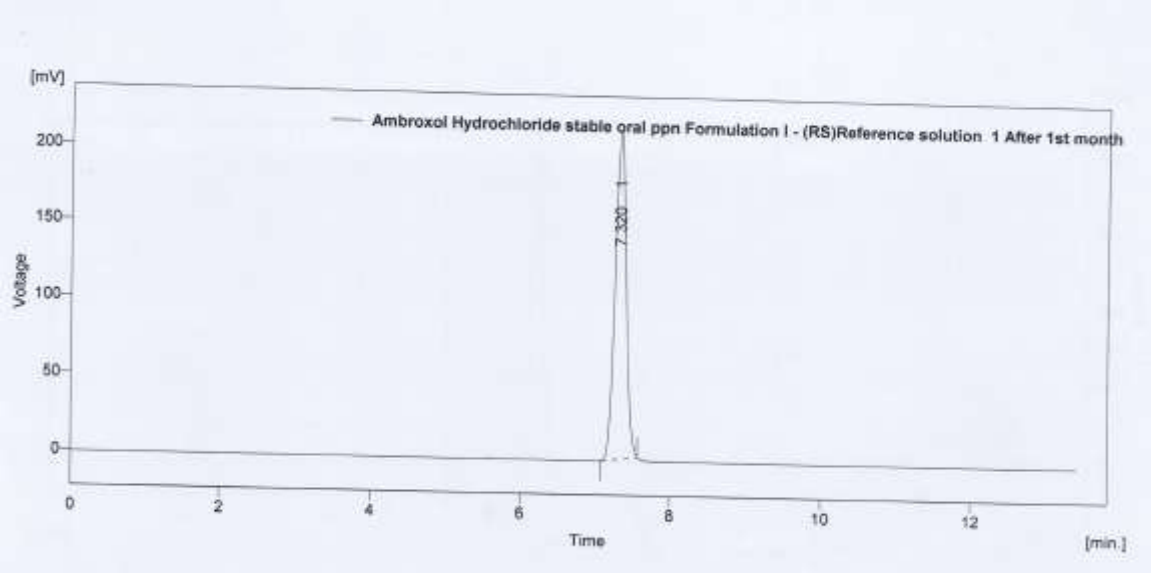


Fig No: 25 HPLC of Test solution-Trial 2 after 1month of stability

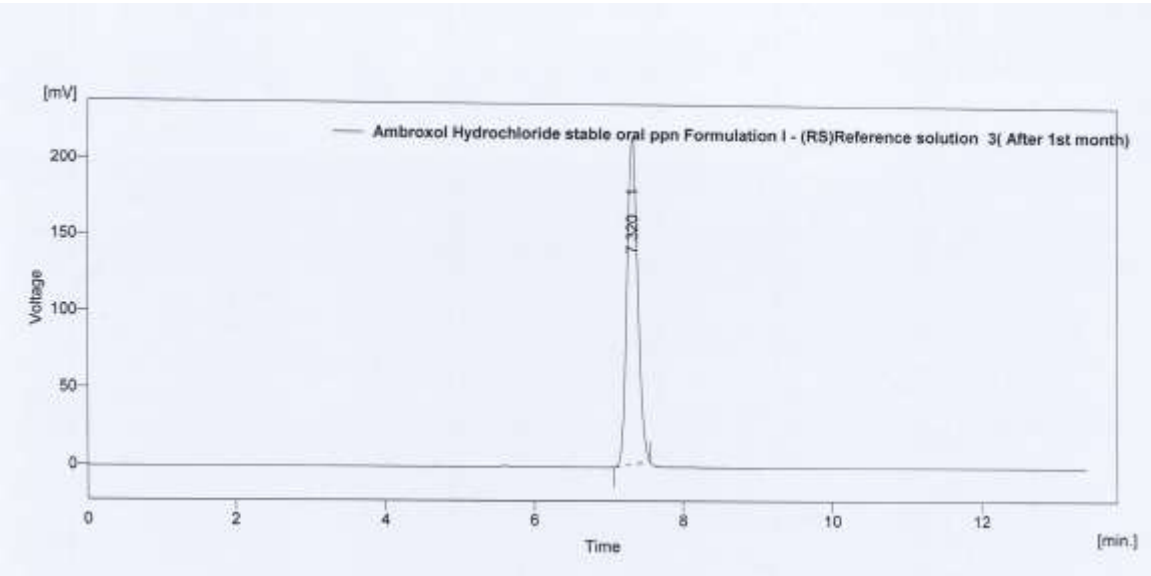


Fig No: 26 HPLC of Test solution-Trial 3 after 1month of stability

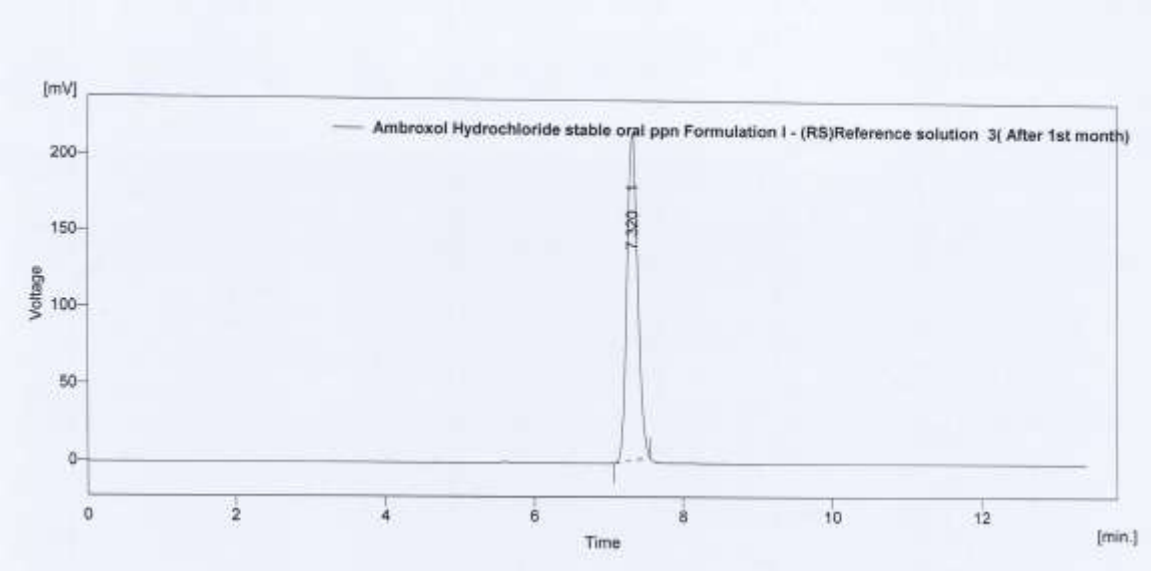


Fig No: 27 HPLC of S1 after 1 month of stability

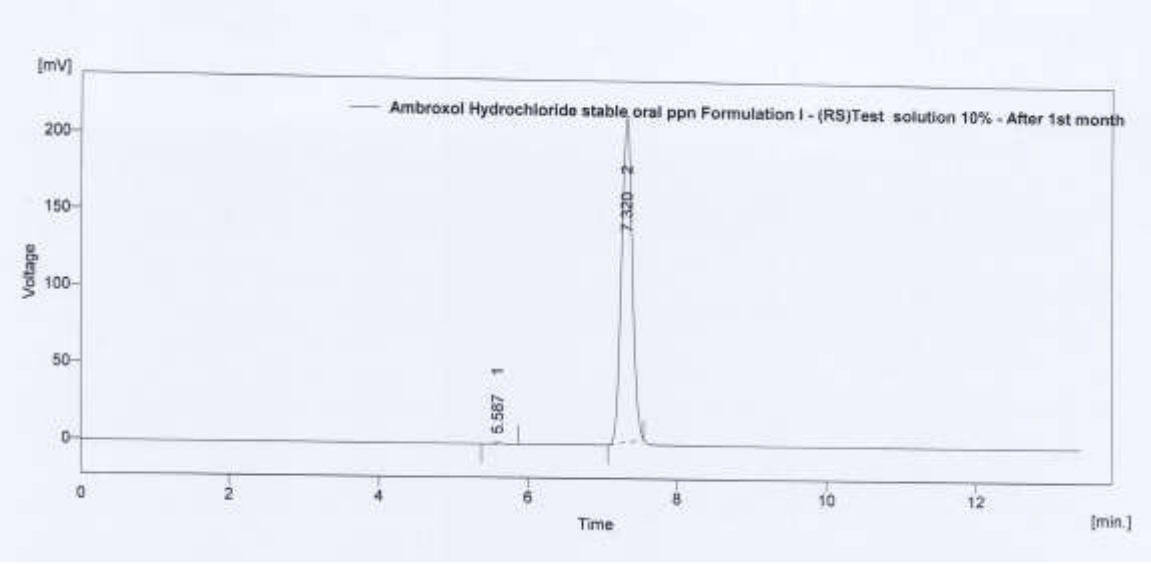


Fig No: 28 HPLC of S2 after 1 month of stability

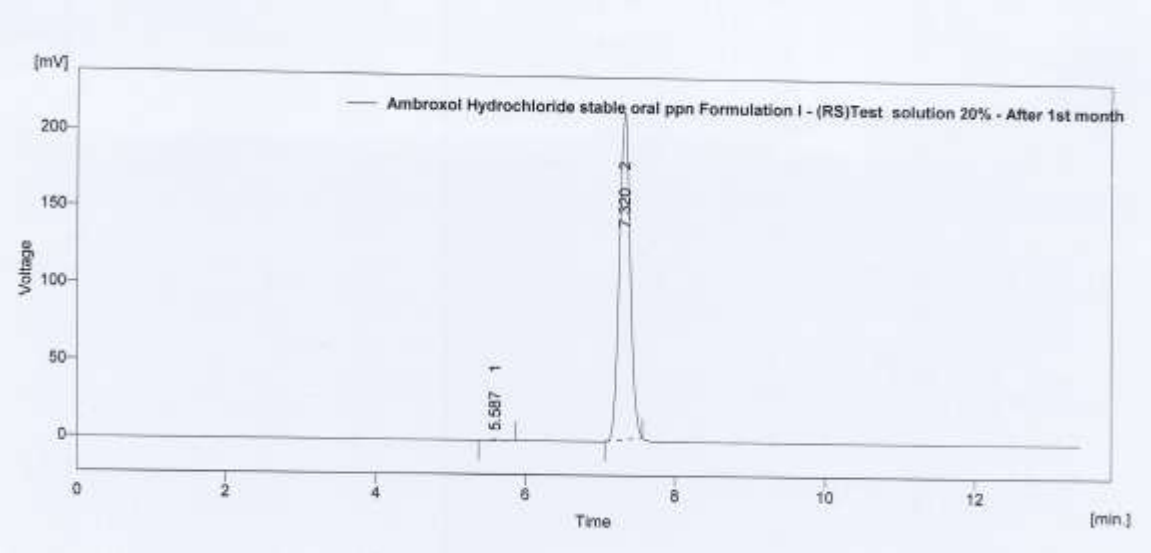


Fig No: 29 HPLC of S3 after 1 month of stability

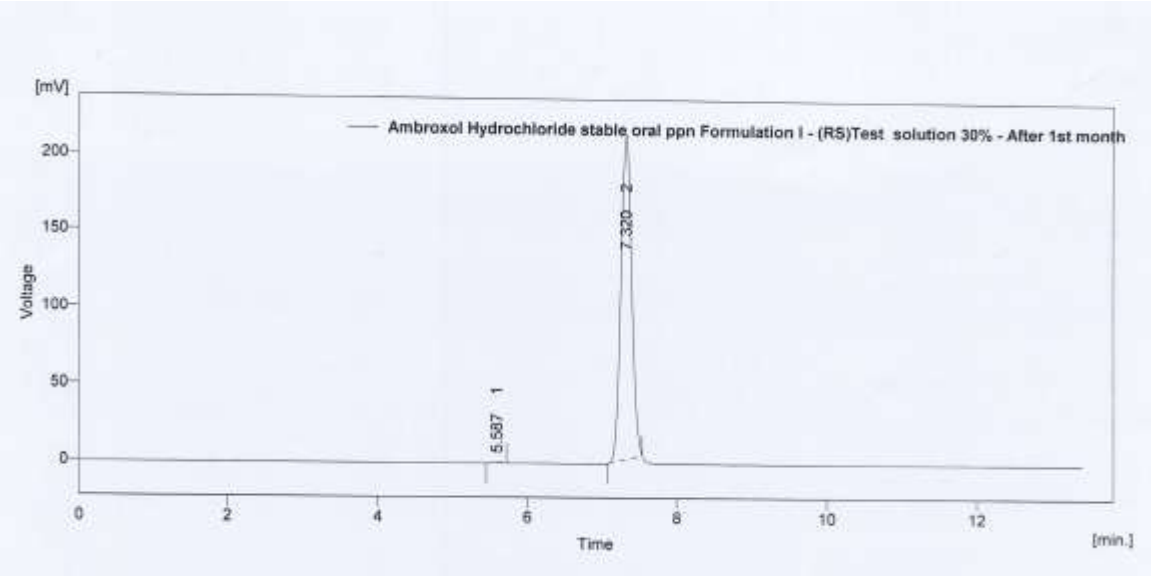


Fig No: 30 HPLC of S4 after 1 month of stability

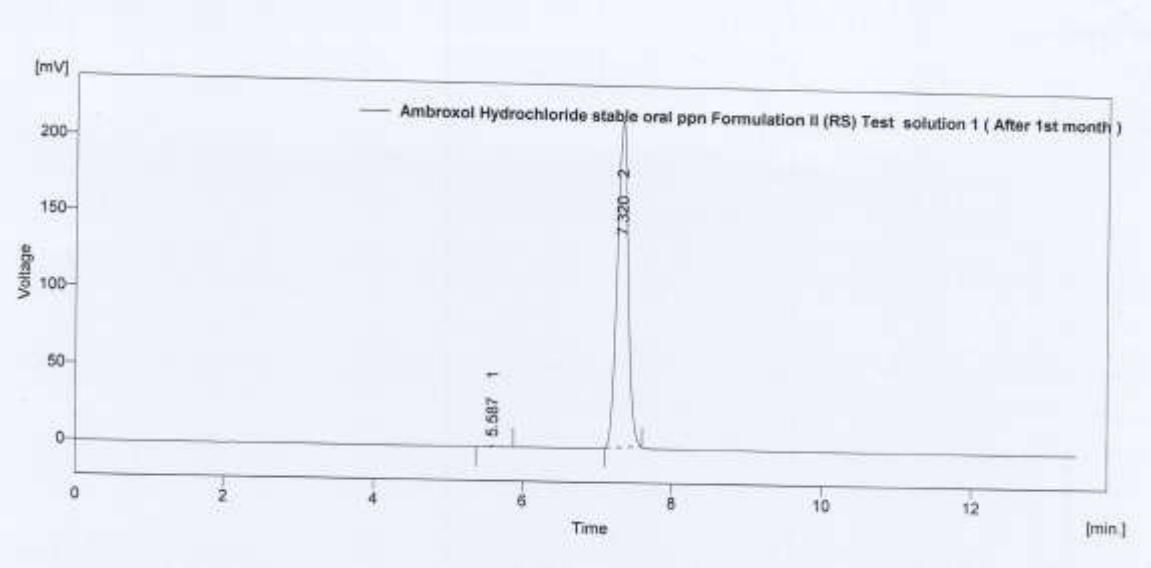


Fig No: 31 HPLC of S5 after 1 month of stability

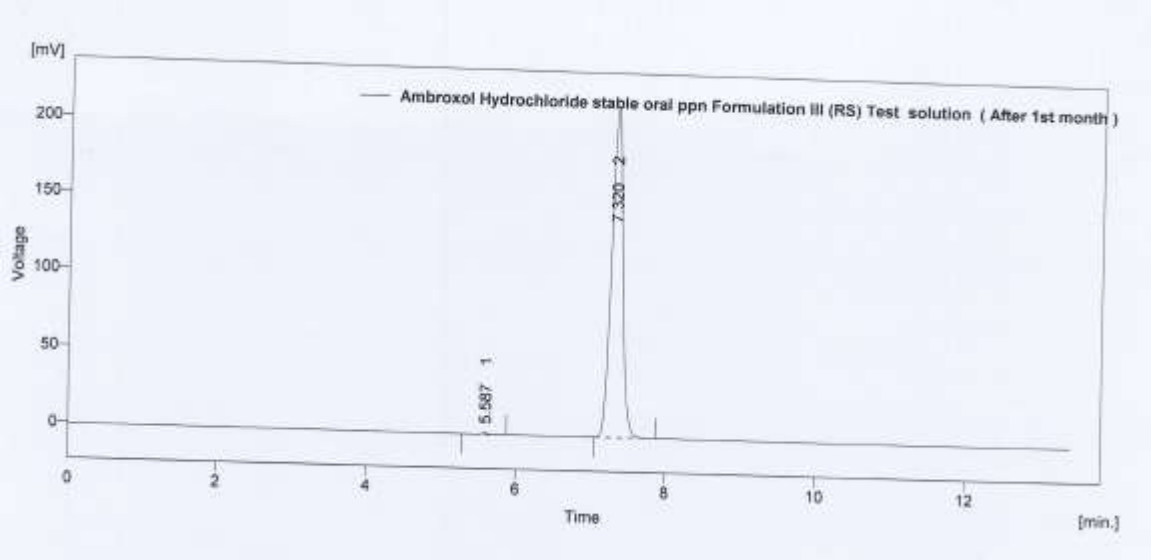


Fig No: 32 HPLC of Test solution-Trial 1after 2 month of stability

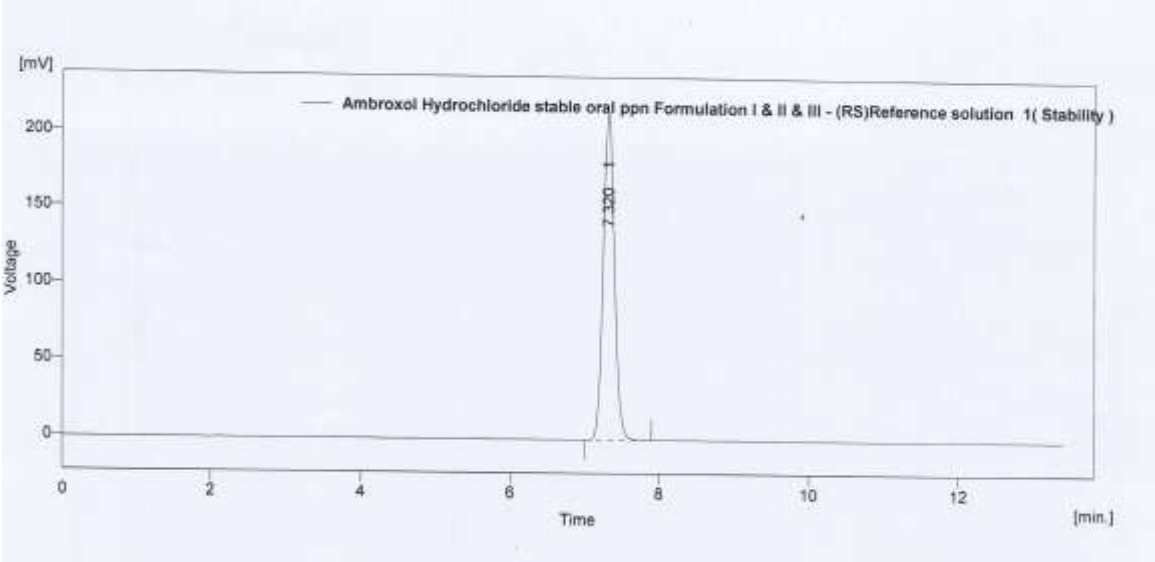


Fig No: 33 HPLC of Test solution-Trial 2after 2 month of stability

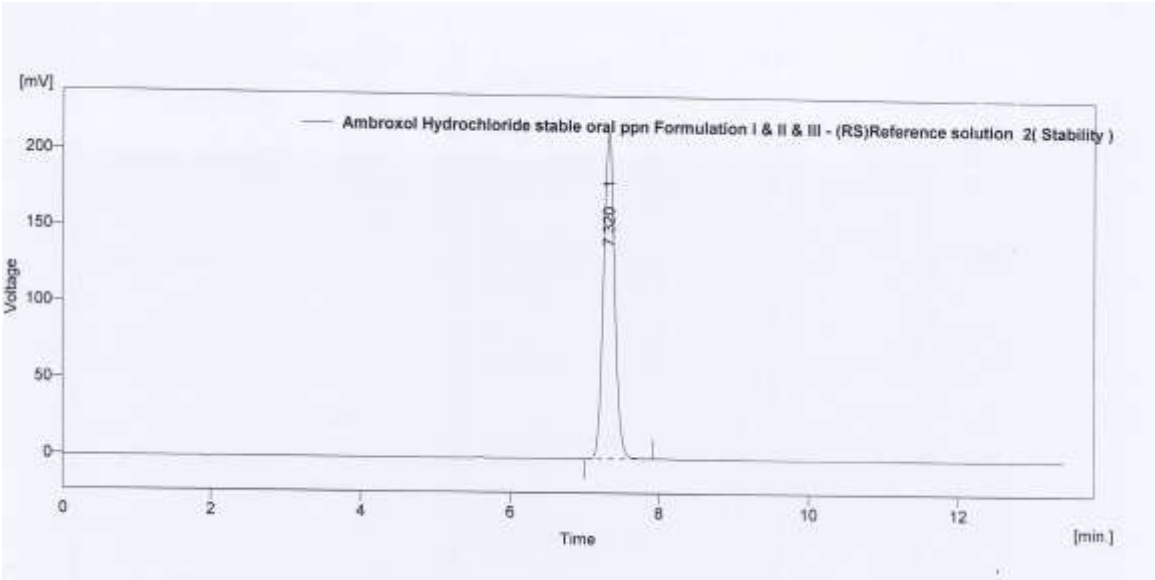


Fig No: 34 HPLC of Test solution-Trial 3after 2 month of stability

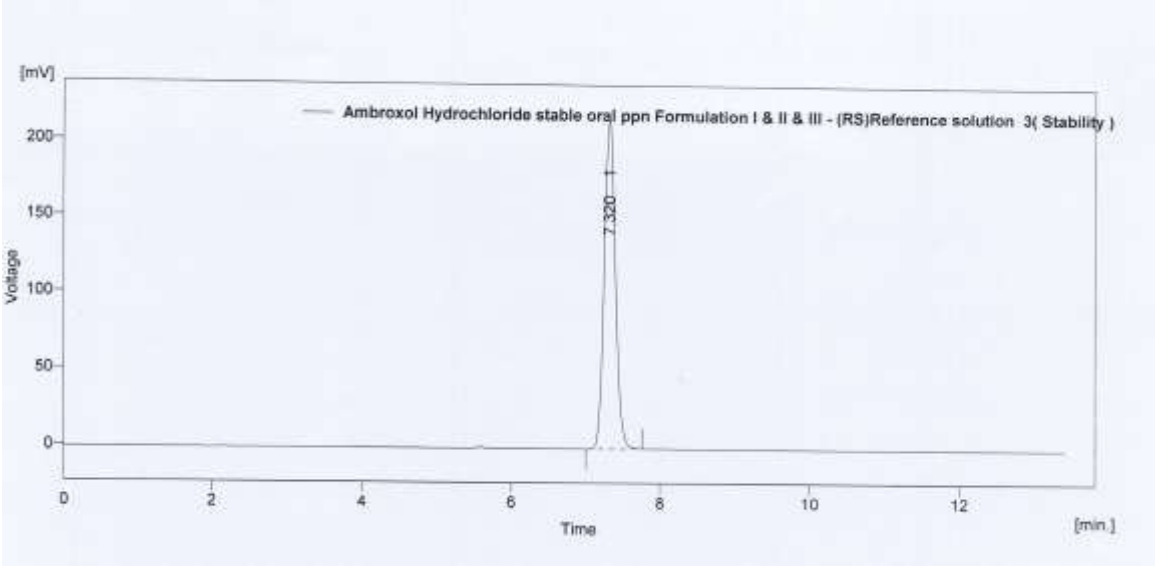


Fig No: 35 HPLC of S1 after 2 months of stability

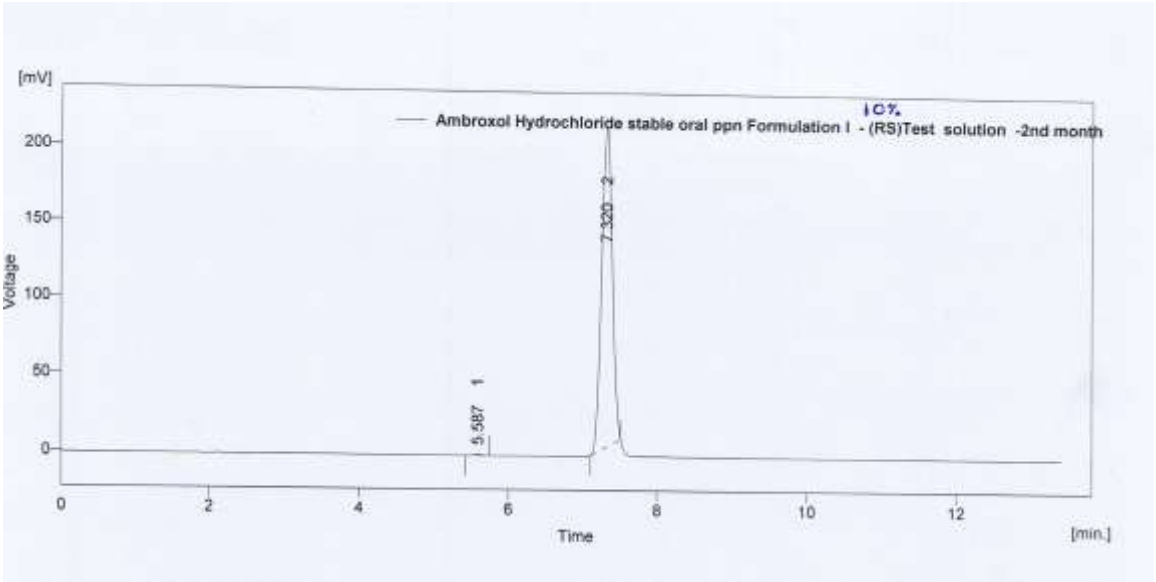


Fig No: 36 HPLC of S2 after 2 months of stability

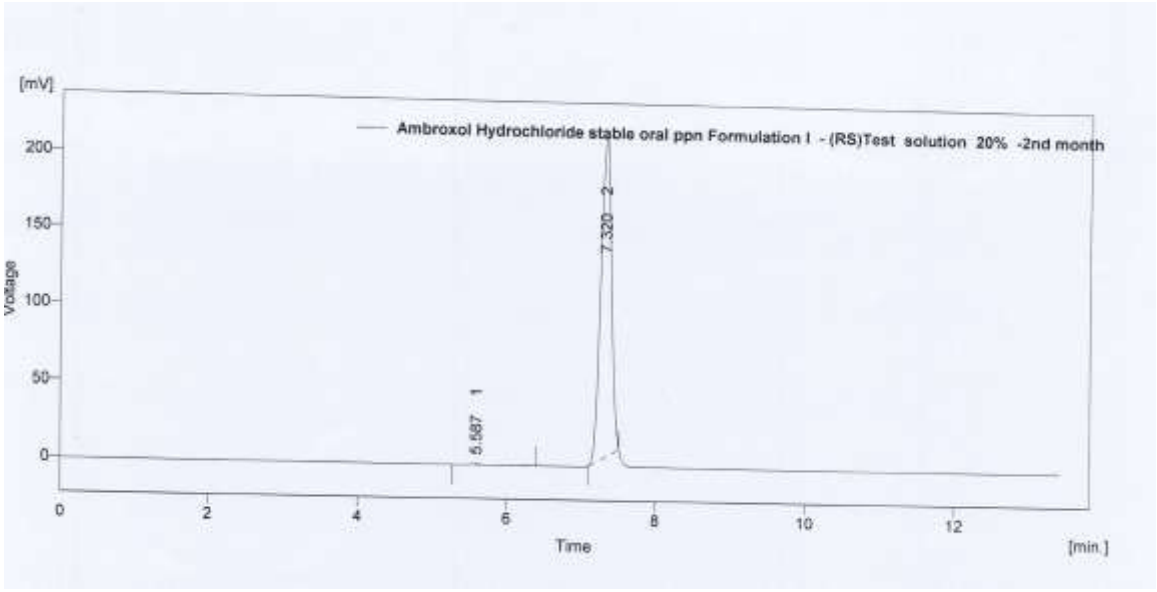


Fig No: 37 HPLC of S3 after 2 months of stability

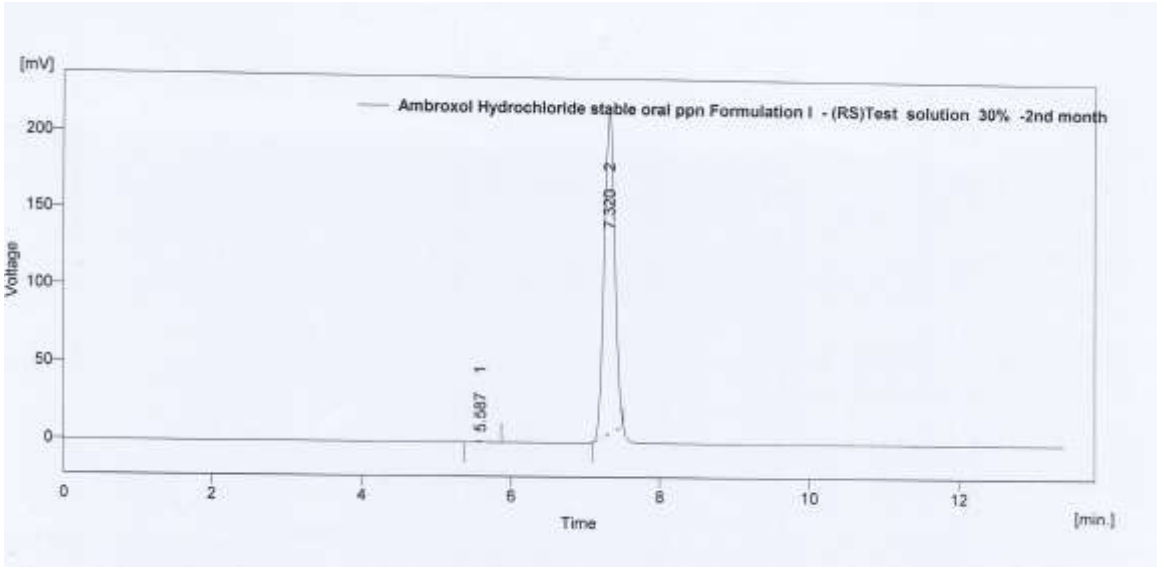


Fig No: 38 HPLC of S4 after 2 months of stability

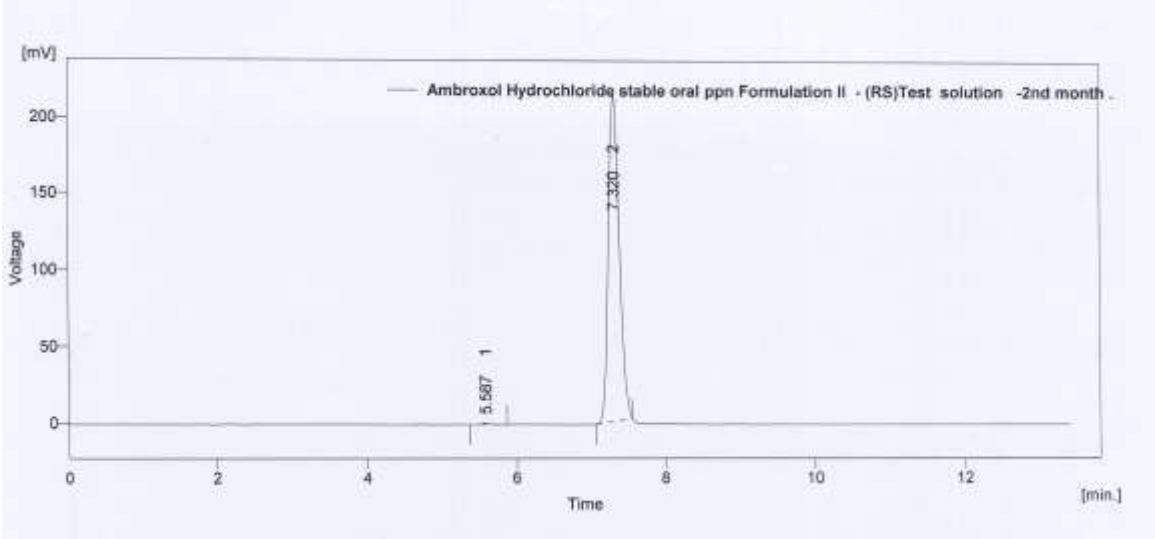


Fig No: 39 HPLC of S5 after 2 months of stability

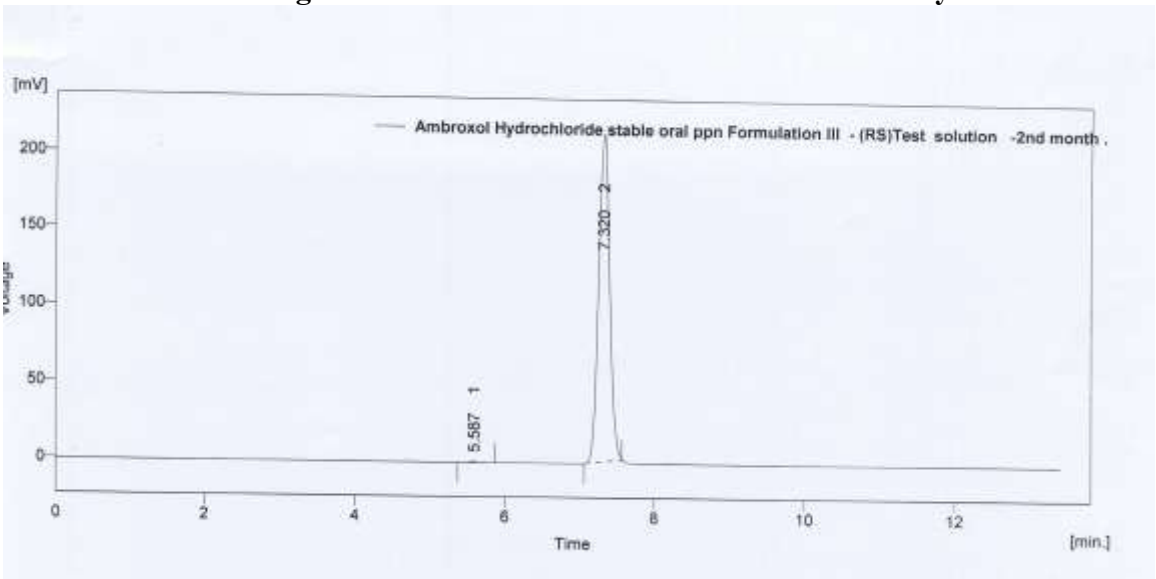


Fig No: 40 HPLC of Test solution-Trial 1 after 3 month of stability

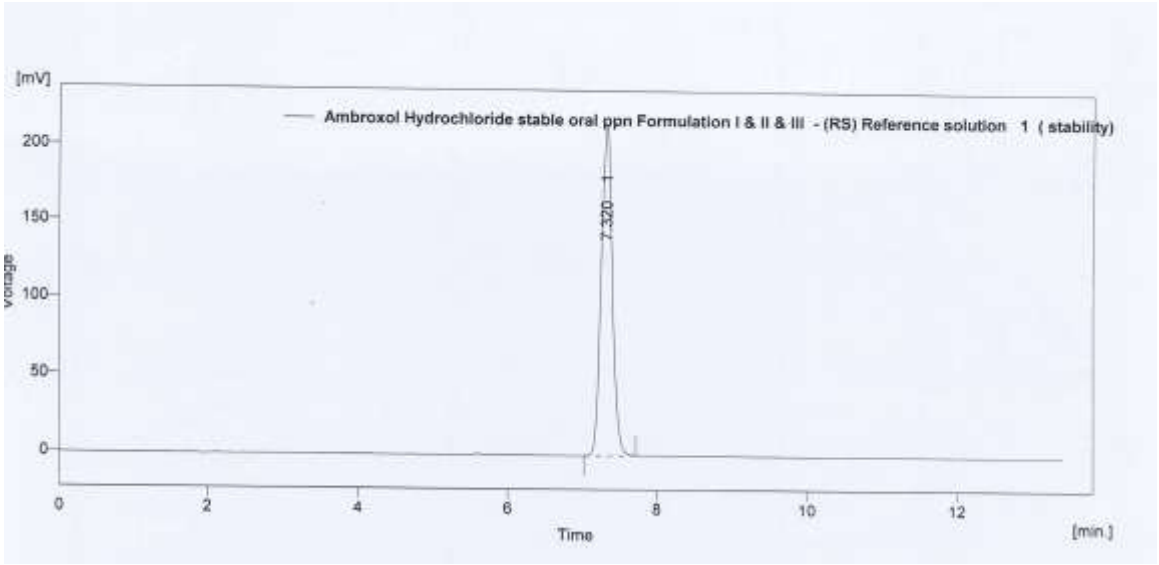


Fig No: 41 HPLC of Test solution-Trial 2 after 3 month of stability

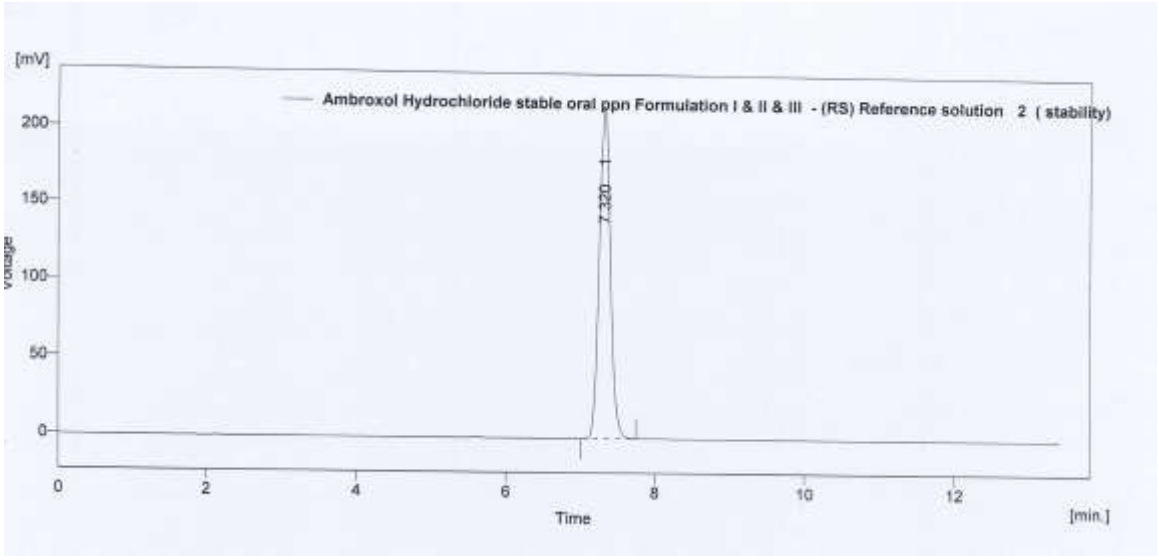


Fig No: 42 HPLC of Test solution-Trial 3 after 3 month of stability

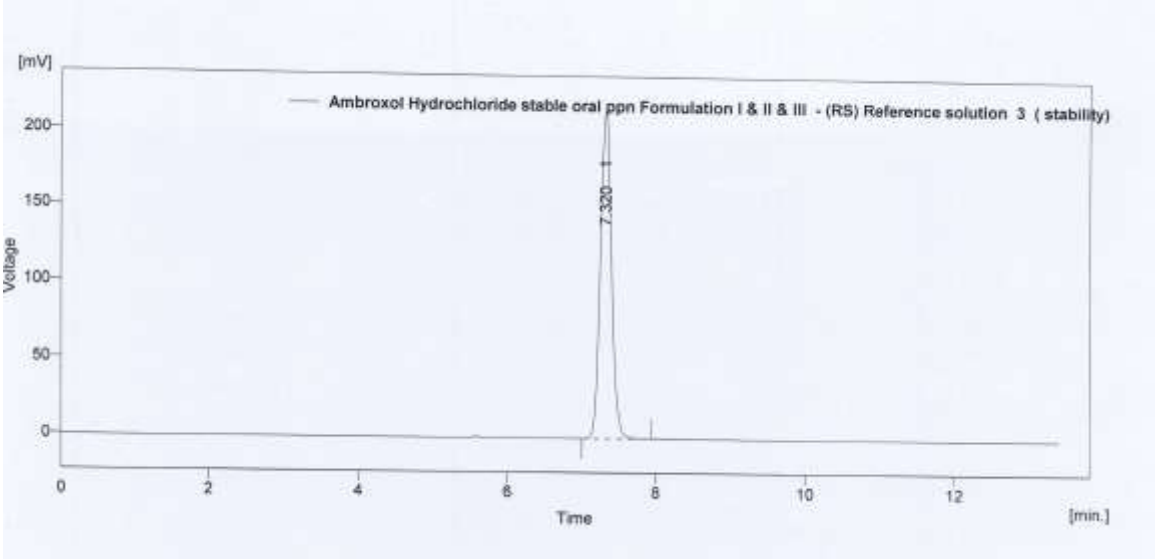


Fig No: 43 HPLC of S1 after 3 month of stability

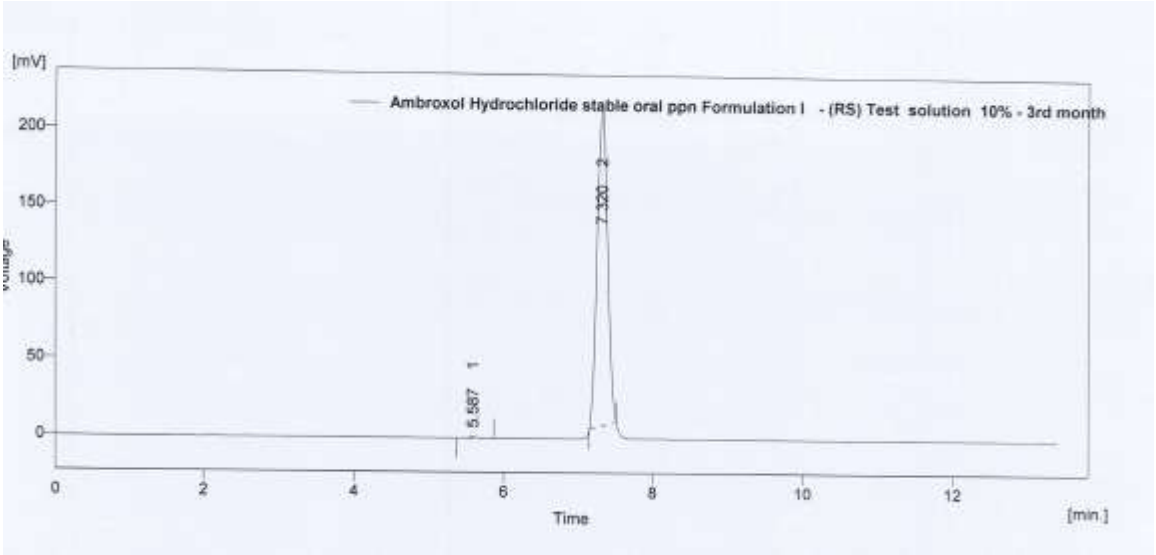


Fig No: 44 HPLC of S2 after 3 month of stability

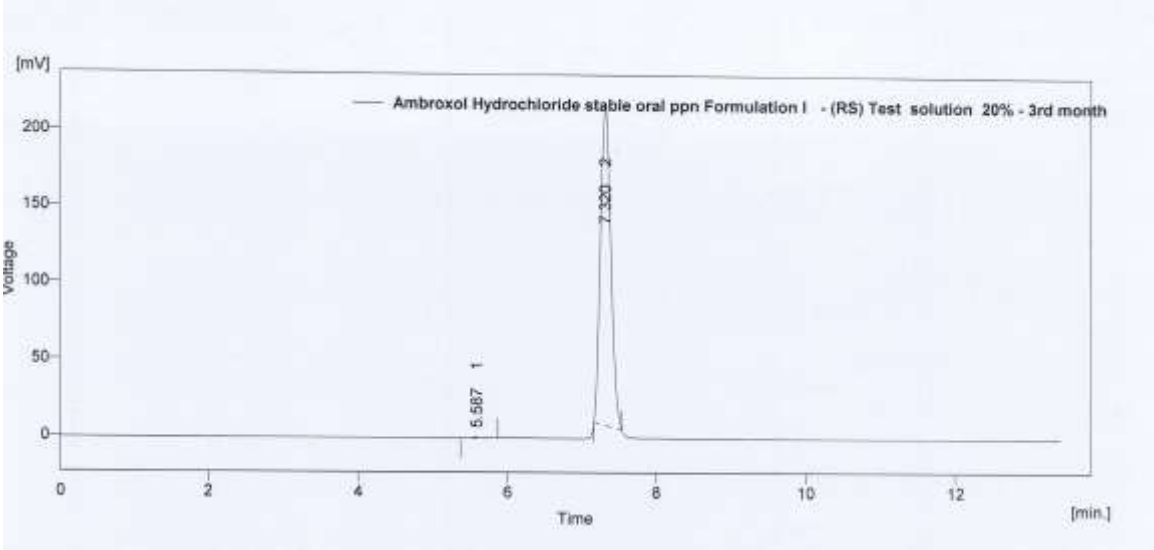


Fig No: 45 HPLC of S3 after 3 month of stability

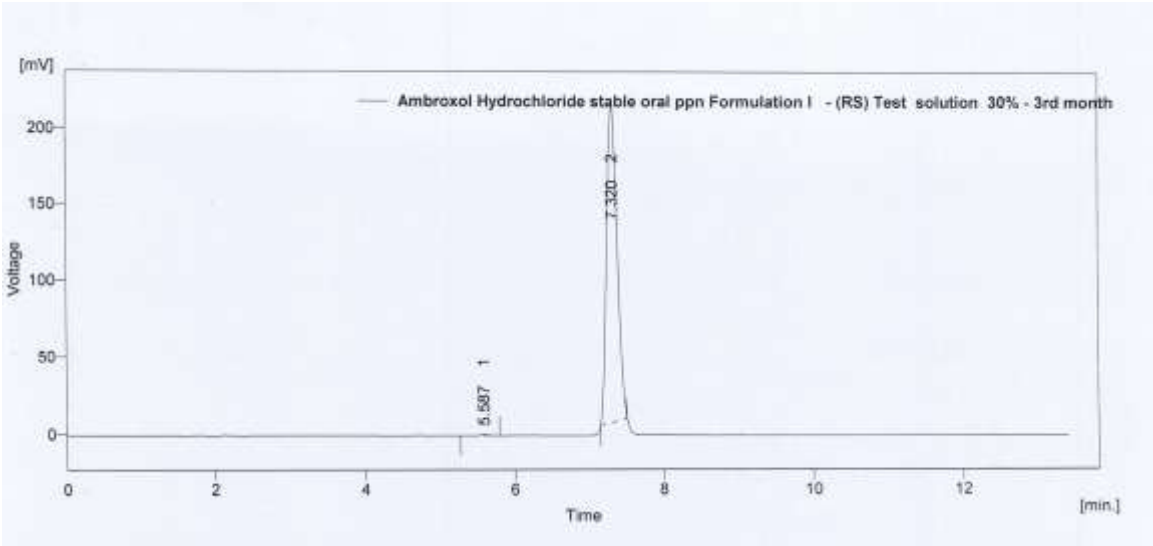


Fig No: 46 HPLC of S4 after 3 month of stability

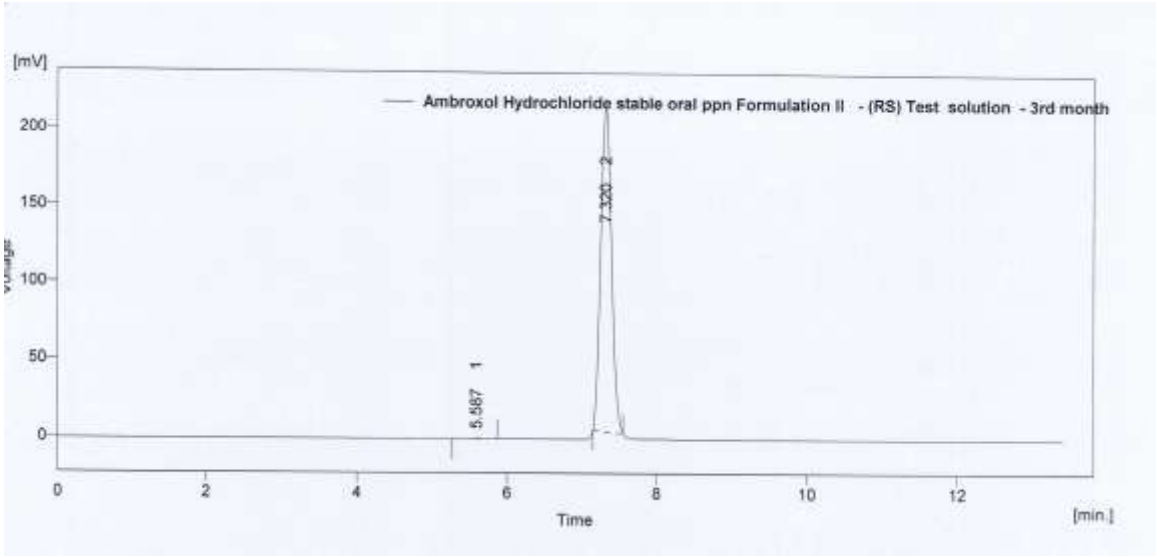


Fig No: 47 HPLC of S5 after 3 month of stability

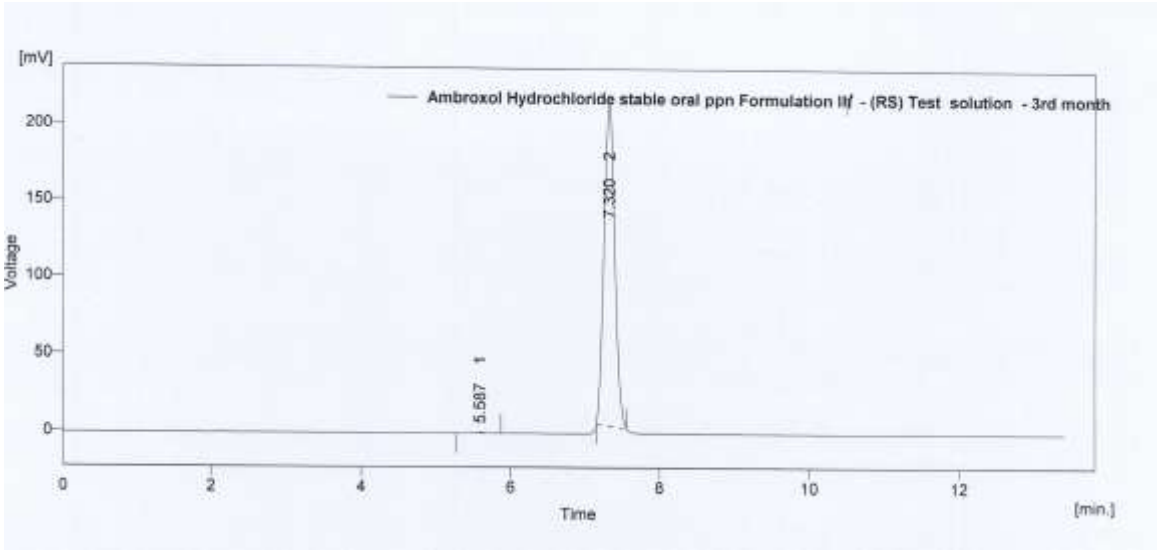


Table No: 21 Stability studies of S1 formulation - Microbial test

TEST MICROBIAL TESTING		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Total viable count	100 CFU/ml	10	20	30	30
Pathogens	should be absent	absent	absent	absent	absent
yeast and mould	not more than 10 /CFU ml	absent	absent	absent	absent

Table No: 22 Stability studies of S2 formulation - Microbial test

TEST MICROBIAL TESTING		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Total viable count	100 CFU/ml	10	20	20	20
Pathogens	should be absent	absent	absent	absent	absent
yeast and mould	not more than 10 /CFU ml	absent	absent	absent	absent

Table No: 23 Stability studies of S3 formulation - Microbial test

TEST MICROBIAL TESTING		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Total viable count	100 CFU/ml	10	20	20	20
Pathogens	should be absent	absent	absent	absent	absent
yeast and mould	not more than 10 /CFU ml	absent	absent	absent	absent

Table No: 24 Stability studies of S4 formulation - Microbial test

TEST MICROBIAL TESTING		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Total viable count	100 CFU/ml	10	20	30	40
Pathogens	should be absent	absent	absent	absent	absent
yeast and mould	not more than 10 /CFU ml	absent	absent	absent	absent

Table No: 25 Stability studies of S5 formulation - Microbial test

TEST MICROBIAL TESTING		STROAGE (IN MONTH)			
		INITIAL	1ST MONTH	2nd MONTH	3rd MONTH
Total viable count	100 CFU/ml	10	10	20	40
Pathogens	should be absent	absent	absent	absent	absent
yeast and mould	not more than 10 /CFU ml	absent	absent	absent	absent

DISCUSSION

Melting point of Ambroxol HCl was found to be 237ºC.

Ambroxol was sparingly soluble in water, slightly soluble in methylene chloride and soluble in ethanol and practically insoluble in ether.

From the UV spectra of the drug it was confirmed that the drug is having maximum absorption at the wavelength of 308nm.

It was found that the solutions of Ambroxol HCl in 0.1N HCl shows linearity (R^2=0.998) in absorbance concentration of 60-140mcg/ml and obey's Beer Lambert's law.

Syrups were formulated in different combinations as S1, S2, S3, S4 and S5

Compatibility studies were done by FT-IR study and it was found that the excipients are compatible with the API.

Different parameters like pH, specific gravity, Viscosity and assay were done for all the formulations and all the values were within the limit.

The formulations were studied for the presence of microbes. All the values obtained were within the limit. Pathogenic organisms were absent.

All the formulations were tested for related substances by HPLC method and no impurities was obtained during the study.

From the release profile the similarity factor was calculated with the marketed syrup solution. The f_2 value of S3 formulation was found to be higher (92.24) when compared to other formulations.

All the formulations were kept for stability study and the parameters such as pH, specific gravity, Viscosity, Related substance and assay were found to be within the limit.

10-30 bacterial colonies were formed in each formulations which was kept for stability study in accelerated condition and it was within the limit.

There was no countable fungal colonies observed in the accelerated conditions.

9. CONCLUSION

From the study which was carried out it was concluded that:

- The S3 batch was selected as the best formulation based on the various evaluation studies.
- The formulation S3 was compared with a marketed sample of Ambroxol hydrochloride syrup and tablet and it was found that the drug release from the formulated syrup matches the drug release profile of the marketed syrup sample. The drug release from the formulated syrup was higher when compared with that of the marketed tablet sample.
- Stability studies also concluded that the drug release profile or other parameters did not alter significantly after the accelerated stability studies.
- Hence a stable ambroxol hydrochloride syrup which had a similarity factor of 93 with the marketed syrup was formulated successfully using sucralose as the sweetening agent.
- Further work can be continued to make formulations with suitable excipients that can prevent crystallisation and to impart latest techniques to mask the bitter taste of the drug.

10 LISTS OF REFERENCES

1. Loyd V Allen, Nicholas G, Popovich, Howard C Ansel. "Ansel's Dosage forms and Drug Delivery Systems". 8th Edition. Wolters Kluwer/Lippincott Williams & Wilkins, 2007:92-96, 100-133, 337.
2. Aulton M E. "Pharmaceutics-The Science of Dosage Form Design". 2nd Edition. Churchil Livingstone, 2003: 309, 321.
3. Remington. "The Science and Practice of Pharmacy". 21st Edition. 2007; Vol. I: 889,929.
4. Rawlins E A. "Bentley's Textbook of Pharmaceutics". Bailliere Tindall, U.K. 2003: 269, 292. B
5. Mithal B M. "The text book of pharmaceutical formulation". Vallabh Prakasan, Delhi; 1980:170-177.
6. Maheshwari R K, Rajagopalan R. "Formulation and evaluation of Tinidazole syrup made by mixed solvency concept technique" Scholars research library 2011, 3(6):266-271.
7. Maheshwari R K, Rajagopalan R. "Formulation and evaluation of Paracetamol syrup made by mixed solvency concept" Scholars research library 2012, 4(1):170-174.
8. Ferreira D C, Morgado R, Bahia M F. "Stability predictions for several expectorant syrups using the accelerated stability programme". EHP 1997; vol.3:157-159.
9. Abdelkawy M, Metwaly F, EI Raghy N, Hegazy M. "Simultaneous determination of Ambroxol hydrochloride and Guaifenesin by HPLC, TLC-spectrodensitometric and multivariate calibration method in pure form and in cough cold formulations". Journal of chromatography and separation technique. 2011:1-12.
10. Stephen childs. "Chemistry of maple syrup". Cornell maple Bulletin 202, 2007:1-4.
11. Patel D A, Patel Yogesh K, Shah P B. "Development and evaluation of herbal syrup from *Neolamarckia Cadamba* (ROXB.) Bosser leaves". International research journal of pharmacy. 2012, 3(9):201-202.
12. Khan H, Zahid Z. "Development and validation of a dissolution test with spectrophotometric analysis Forgemifloxacin mesylate and Ambroxol hydrochloride in tablet dosage form. International Journal of PharmTech Research 2012; vol.4(2): 661-668.
13. Joseph H, Bruemmer, Alice P B. "Storage stability of orange syrups". Proc.Fla.State Hort.Soc.90, 1977: 183-185.

14. Abdul A S, Venkateswaralu Y, Umal Khair S. "Modified release capsules of Ambroxil, Preformulation and evaluation. 2011,2(3): 1-19.
15. Mizanur R M, Mohammad M F, Shahid-Ud-Daula. "*In-vitro* release kinetics study and optimization of Ambroxil HCl 75mg matrix tablets using response surface methodology". International research journal of pharmacy, 2012,3(5):165-172.
16. Brooks A A, Asamudo N U, Takon I. "Bacteriological stability of high sugar pediatric syrups probability of survival of contaminating bacteria". Journal of pharmaceutical and biomedical sciences. 2012, 22(13):1-4.
17. Priya C J, Tushar S L, Manali W J, Pallavi P Y, Ishita P. "Taste masking of Ambroxol hydrochloride by formulating and evaluation of microsphere by using spray dryer". International journal of pharmaceutical research and development. 2012: 81-85.
18. Anbu Jeba Sunilson1 J, Anandarajagopal K, Abdullah khan, Khaja Pasha, Qusro Bin Hassan, Puspa V. Kuna Raja. "Antihistaminic evaluation of formulated polyherbal cough syrup". Journal of Medicinal Plants Research, 2010 Vol. 4(14):1482-1485.
19. "Indian Pharmacopoeia", Vol II; Controller of Publications, Ministry of Health. 2007: 701.
20. Rang H P, Dale M M, Ritter J M, Flower R J. "Rang and Dale's Pharmacology". 6th Edition. Churchill Livingstone: 278-279.
21. Laurence L Brunton, John S Lazo, Keith L Parker. "Goodman & Gilman's The Pharmacological Basis of Therapeutics". 11th Edition. McGraw-Hill, 2006: 559-572.
22. Tripathi K D. "Essentials of Medical Pharmacology". 5th Edition. Jaypee Brothers, New Delhi, 2003: 195,196.
23. Raymond C Rowe, Paul J Sherskey and Sian C Owen. "Handbook of Pharmaceutical Excipients". 5th Edition. Pharmaceutical Press, 2006: 132-135, 278-282, 430-432, 767-769, 821-823.
24. "The British Pharmacopoeia" Market towers, 2009: 265-268.
25. Leon Lachman, Herbert A Lieberman. "The theory and practice of Industrial Pharmacy". Special Indian Edition. CBS Publishers and Distributors, 2010: 293-335, 346-371.
26. Adjei A, Doyle Reiland T, Swarbrick J, Boylan J C. "Encyclopedia of pharmaceutical technology". Marcel Dekker, New York; 1992:117. Sharma Y R. "Elementary organic spectroscopy". S.Chand & Company Ltd, 2010: 93-117.
27. Gurdeep R Chatwal, Sham K Anand. "Instrumental methods of Chemical Analysis". 5th Edition. Himalaya Publishing House, 2010: 2.44, 2.55-2.75.

28. Vijayaragavan C. “A practical handbook of physical pharmaceutics” 1997: 23-25.
29. Guruprasad M, Prabal Kumar M. “Physical pharmacy A practical text”. 1998: 12-16.
30. “Indian Pharmacopoeia”, Vol I; Controller of Publications, Ministry of Health. 2007: 36-40.
31. Tushar S L, Manali W J, Pallavi P Y, Ishita P. “Taste masking of Ambroxol hydrochloride by formulating and evaluation of microsphere by using spray dryer”. International journal of pharmaceutical research and development. 2012: 81-85.
32. Dachinamoorthi D, Venkateswaramurthy N. “Biopharmaceutics and pharmacokinetics- A practical Manual”. PharmaMed press. 2010:15-17.
33. Brahmankar D M, Sunil B Jaiswal. “Biopharmaceutics and pharmacokinetics A Treatise”. Vallabh Prakashan. 2009: 331-333.
34. http://o.tqn.com/
35. http://www.chemicalbook.com/
36. http://www.chemspiders.com/
37. http://chemistry.about.com/